Effect of Fried Lard and Corn Oil on Blood Cholesterol in Hamsters

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Abstract

Frying is one of the most common methods to prepare foods in restaurants, food industries, and home kitchen. During frying, oil undergoes extensive physical and chemical changes, resulting in color darkening, foaming, hydrolysis, oxidation and polymerization. For cost effectiveness, heated fats/oils in restaurants are used for up to 1 week before they are discarded and replaced. It is known that saturated fatty acid consumption elevates blood cholesterol level and increases the risk of coronary heart disease, while the polyunsaturated fatty acid consumption decreases blood cholesterol by up-regulation of LDL receptors. It is believed that consumption of lipid peroxidation products from frying oil/fats contributes to the pathogenesis of atherosclerosis. However, to date, no research has examined effect of fried lard and fried corn oil on blood cholesterol level compared with that of non-fried oils.

The present studies used Golden Syrian hamsters as an animal model to investigate effect of fried versus non-fried lard and corn oil on blood cholesterol level. In brief, 45 male hamsters were divided into 4 groups (n=11~12 each) and fed one of four diets containing 0.1% cholesterol with addition of 10% non-fried oil and 10% fried oils heated for 2,4,6 days at 180°C with 9 hr frying per day. The experiment groups were named as non-fried lard (NFL), fried lard for 2 days (2FL), for 4 days (4FL), and for 6 days (6FL). Similarly, NCO referred to a group of hamsters fed non-fried corn oil while 2FCO, 4FCO and 6FCO represented three groups of hamsters fed the diet containing 10% corn oil which was heated for 2, 4

and 6 days, respectively.

The present data demonstrated that hamsters had a decreased level of total serum cholesterol (TC) and HDL-cholesterol (HDL-C) when they were fed the diets containing fried-lard compared with the control group ($P<0.05$, $P<0.005$ respectively). When corn oil was used, an increased TC level was found in 4FCO and 6FCO groups compared with 2FCO and NCO at wk 3 ($P<0.05$). Afterwards, the difference in serum TC became insignificant at wk 6. Due to such alternation, serum obtained at wk 7 was also determined. The TC of 4FCO and 6FCO was significantly lower than that of the control group. In both experiments, the groups which ingested food that contained oil fried for 4d and 6d had significantly lower hepatic and renal cholesterol level when compared with the control group. And it was consistent in both experiments that the HDL-C was much lower in groups consumed fried oil.

Feeding fried corn oil led to an increase in serum triglycerides (TG) ($P<0.01$). Although there was no difference in serum TG level in fried lard experiment, the hepatic TG level of the groups that were fed on fried oil were significantly lower than that of the control group, respectively. And the free fatty acid (FFA) content in the liver tended to increase with the time of frying treatment. Nevertheless, there was no difference in live phospholipids (PL) concentration in fried corn oil experiment, but 6FL gained a much lower phospholipids content when compared with the other three groups.

Up to date, researches have found out that the thermoxidized oil contains

lipid peroxidation product, such as hydroxy- and hydroperoxy fatty acids and cyclic fatty acids, all of which have been shown to be potent PPARα activators. The activation of PPARα has been proved that it could lead to a reduced transcription of HMG-CoA reductase and LDL receptor, which could partly explain the reduced serum HDL cholesterol level and hepatic cholesterol level in experimental groups when compared to the control groups. But the underlying mechanism is still under investigation by further experiments.

摘要

油炸是酒店、食品工業以及居家最常見的烹飪方法之一。在油炸過程中，食用油會發生大量的物理及化學反應，致使產生顏色加深、起泡、脫水、氧化及多聚化等現象。從成本角度考慮，酒店通常會將煎炸食用油類使用一個星期左右之後才會丟棄。眾所周知，攝入過多飽和脂肪酸會升高血液膽固醇水平同時增加冠心病風險。然而多不飽和脂肪酸則會通過提高低密度脂蛋白受體活性從而降低血液中總膽固醇含量。通常認為，油炸用油中產生的酯類多氧化合物的對動脈粥樣硬化的形成有所協助。但是到目前為止，尚未有相關研究探索用於煎炸用途的豬油和玉米油對於血液中膽固醇的影響。

本研究使用金黃倉鼠作為實驗對象，對比非油炸以及油炸豬油和玉米油對血液膽固醇水平的作用。實驗分兩次進行。簡單來說，45隻雄性倉鼠分為4組（每組11至12只），並餵食含有0.1%膽固醇以及10%非油炸油或10%油炸油的定食（這些油在180攝氏度每天加熱9小時，分別加熱2，4和6天）。實驗對照組由食用非油榨油的倉鼠組成，食用分別含有油炸兩天、四天及六天的豬油定食的倉鼠為實驗小組。同樣，油炸玉米油實驗也如此分組。

實驗數據表明，在實驗開始第三週以及第六週，食用油炸豬油定食的倉鼠血清總膽固醇水平以及高密度脂蛋白中膽固醇的含量較對照組有明顯降低。而當餵食玉米油時，第三週時，食用油炸了四天以及六天的玉米油定食的倉鼠血清中總膽固醇量較其餘兩組明顯增高，但這個變化到了第六週時被組間相差

無幾的血清總膽固醇含量所替代。由於發生了該變化，第七週時抽取的血清也被用於測試，結果表明，在第三週血液總膽固醇明顯升高的兩組在第七週的總膽固醇含量明顯低於對照組。兩組實驗中，食用含有油炸四天以及六天的小組的肝內以及腎內膽固醇含量較同實驗下其餘兩組的要低。相似的，在兩組實驗中，食用油炸油定食的小組都較對照組有著顯著更低的高密度脂蛋白膽固醇。

食用油炸玉米油導致實驗組倉鼠的血清三酸甘油酯含量明顯高於對照組。雖然用豬油作實驗用油的實驗中，各小組間的血液三酸甘油酯含量相差無異，但是相似的是，兩組實驗中，實驗組的肝內三酸甘油酯含量都明顯低於對照組，而自由脂肪酸含量隨著油炸時間呈遞增趨勢。肝臟中磷脂含量在玉米油實驗中組間沒有差異，使用豬油的實驗中，食用油炸六天定食的小組的肝內磷脂濃度較其餘三組都要低。

目前而言，有研究表明，加熱氧化的油裡面含有大量羥基，過氧羥基脂肪酸， 以及環氧脂肪酸。這些脂類過氧化產物已被證明是強有力的PPARα活化因子。PPARα通路的活化已被證明可以導致HMG-CoA還原酶和低密度脂蛋白受體在轉錄水平的降低，從而可以部分地解釋本實驗中食用油炸油小組裡肝內膽固醇和高密度膽固醇相對對照組偏低的結果。而真正的原因還有待進一步實驗考證。

List of Abbreviations

ACAT	acyl coenzyme A cholesterol acyltransferase
ACC	acetyl-CoA carbooxylase
ANOVA	analysis of variance
APOA1	apolipoprotein A1
BF3	boron trifluoride
CHD	coronary heart disease
CM	chylomicron
COPs	cholesterol-oxidized products
CYP7A1	cholesterol 7α-hydroxyase
ER	endoplasmic reticulum
ERs	estrogen receptors
FFA	free fatty acids
FAS	fatty acid synthase
FXR	farnesoid X receptor
G6PD	glucose-6-phosphate dehydrohenase
HDL	high-density lipoprotein
INSIG	insulin-induced genes
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol

LPL	lipoprotein lipase
LXR	liver X receptor
NCO	non-fried corn oil
NFL	non-fried lard
PL	total phospholipids
POV	peroxide values
PPARs	peroxisome proliferator activated receptors
RCT	reverse cholesterol transport
S1P	site-1 protease
S2P	site-2 protease
SCAP	SREBP cleavage-activating protein
SREBP	sterol regulatory element-binding protein
SD	standard deviation
TC	total cholesterol
VLDL	very low-density lipoprotein
VSMC	vascular smooth muscle cells

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Chapter 1

Introduction

1.1 Frying

Fats and oils are a major component in the human diet. Their importance lies in their high energy content. In addition, they provide essential fatty acids and act as carriers for fat-soluble vitamins. Besides its nutritional value, fat also has other important properties. As a sort of good flavor carriers, fats provide succulence and mouth-feel to a wide range of foods. To meet different needs of food preparations, fats and oils undergo various procedures such as hydrogenation, fractionation, blending and emulsification etc., after which they are applied as spreadable products, bakery margarines, mayonnaise, chocolate and ice cream fat source, biscuit fats and frying media (Podmore, 2000).

1.1.1 General introduction of frying

Frying is one of the fastest, oldest and simplest methods of food preparation. It is useful in processing almost all kinds of food, including meat, fish, vegetables, mushrooms and seafood and is popular throughout the world in domestic, restaurant and industrial establishments. Frying make it fast to cook the food through to the middle while in the mean time generates a distinctive flavor-texture combination to the food. Such features meet the need for efficiency of industries and make the frying food irresistible to the worldwide customers for years.

There are usually two types of frying methods, shallow frying and deep-fat frying. Shallow frying, also known as pan-frying, generally means the oil is used only once, so that the resistance of the oil to oxidative breakdown is not important. The oil has only a minor role as a heat transfer medium and its action is to prevent the food from sticking to the pan and to give the food an oily glaze (Perkins *et al.*, 1996). Besides, it may also contribute to the finish-flavor with spices that processed in it in typical Chinese cooking. The oils that used must either have a clean tasting and completely bland, or they must be highly characteristic and so complement to food flavor, as in the case of oils such as olive oil and sesame oil, as well as butter and margarine.

In deep-fat frying, the oil or the fat acts as heat transfer medium which therefore exposed to high temperatures while the food that processed is partially or totally submerged. In addition to the changes in the food that brought about by the heat, numerous chemical reactions take place thus changes the physical nature of the fat itself as well as the characteristic of the final product (Moreira, 1999b).

1.1.2 Physical and chemical changes of oils during frying

1.1.2.1 Physical changes

Frying is probably the most complex food processing operations because of the multiplicity of reactions that occur and the vast quantity of chemical products that are generated (Stauffer, 1996). During deep-frying, heat is transferred from the oil to the food, while water is evaporated from the food and oil is absorbed by the food as shown in **Figure 1.1**. Physical changes in oils that occur during heating and

frying include increased viscosity, color darkening, increased foaming and decreased smoking point (Moreira, 1999b). Even though methods have been set up to measure these physical changes quantitatively, they are usually evaluated subjectively by visual inspection during practical frying, which are often used by restaurants as one of the aspects to determine when to discard the frying oils. The relationship of the physical and chemical changes in oil is usually predictable because the decomposition products from the chemical breakdown of the oil cause the physical changes in frying oils, such as increase in viscosity, color, foaming and the pungent and burnt odors they give out.

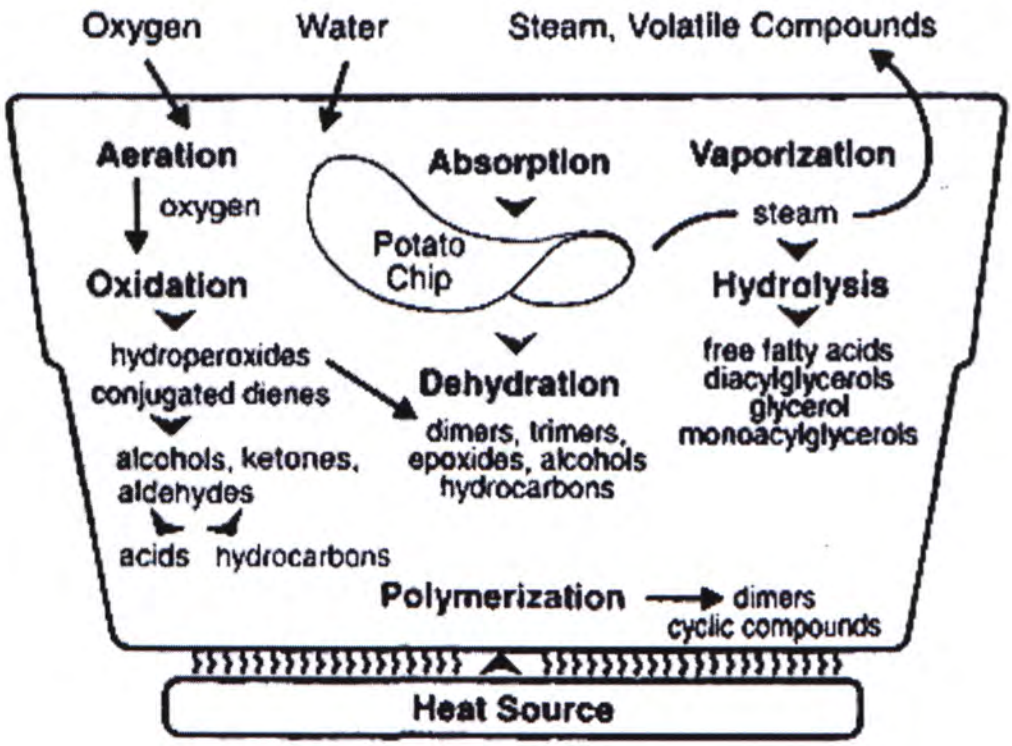


Figure 1.1 Physical and chemical reactions that occur during frying.

Adapted from Warner (Warner, 2004)

1.1.2.2 Chemical changes

During deep-frying, various deteriorative chemical reactions take place, such as hydrolysis, oxidation and polymerization, and oils decompose to form volatile products and nonvolatile monomeric and polymeric compounds. The deterioration of the fat at elevated temperatures is influenced by various factors, e.g. the nature of the cooking fat, the condition of the frying, the kind of heat transfer, the use of antioxidants and other additives (Stauffer, 1996).

1.1.2.2.1 Hydrolysis

Triglycerides in the oil are hydrolyzed by water and steam, which produces monoacylglycerols and diacylglycerols, and eventually free fatty acids (FFA) and glycerol (**Figure 1.2**). Glycerol will partially evaporate because it volatilizes at temperature above 150 °C. And the reaction equilibrium is shifted in favor of other hydrolysis products. FFA, monoacylglycerols and low-molecular-weight acidic products produced from frying enhance hydrolysis in the presence of steam during frying. Degradation products from hydrolysis decrease the fry life of the oil and the level of FFA increases with increase time of frying (Naz *et al.*, 2005).

1.1.2.2.2 Oxidation

Oxygen, pre-existed in frying oils or added with food placed in the fryer, together with the heat of the oil, produces a series of reactions including formation of free radicals, hydroperoxides and conjugated diene. The chemical reactions that occur during the oxidation process help to form both volatile and nonvolatile decomposition compounds. The oxidation mechanism in frying oils is similar to

autoxidation at room temperature. However, the unstable primary oxidation products, *viz* hydroperoxides, decompose quickly at frying temperatures into secondary oxidation products, such as aldehydes and ketones (**Figure 1.3**). Secondary oxidation products that are volatile contribute significantly to the odor of the oil and flavor of the fried food. Analysis of primary oxidation products, at any point in the frying process could provide with little information because their formation and decomposition fluctuate rapidly and are not easily predicted. During frying, oil with PUFA have a distinctive induction period of hydroperoxide formation followed by a rapid increase in peroxide values (POV), after which a rapid destruction of peroxides (Warner, 2004). Measuring levels of PUFA can help determine the extent of thermal oxidation. Oxidative degradation will produce oxidized triacylglycerols containing hydroperoxide-, opoxy-, hydroxy- and keto-groups and dimeric fatty acids or dimeric TG (Kubow, 1992). Together with hydrolysis, the pre-existed cholesterol in animal fats that used for frying may form cholesterol-oxidized products (COPs) (Kubow, 1992; Rao, 1996). There are more than sixty known COPs (Osada *et al.*, 1993), which have been reported to have a wide range of adverse adverse biological effects on animals, such as atherogenesis, cytotoxicity, mutagenesis and carcinogenesis (Guardiola *et al.*, 1996).

1.1.2.2.3 Polymerization

Polymerization results in the formation of compounds with high molecule weight and polarity (**Figure 1.4**). Polymers can form from free radicals or triacylglycerols by the Diels-Alder reaction. The Diels-Alder reaction is an organic chemical reaction (specifically, a cycloaddition) between a conjugated diene and a

substituted alkene, commonly termed the dienophile, to form a substituted cyclohexene system (Diels *et al.*, 1928). Cyclic fatty acids can form within one fatty acid; dimeric fatty acids can form between two fatty acids, either within or between triglycerides; and the polymers with high molecular weight are obtained as these molecules continue to cross-link. As polymerized products increase in the frying oil, the viscosity of the oil increases as well (Warner, 2004).

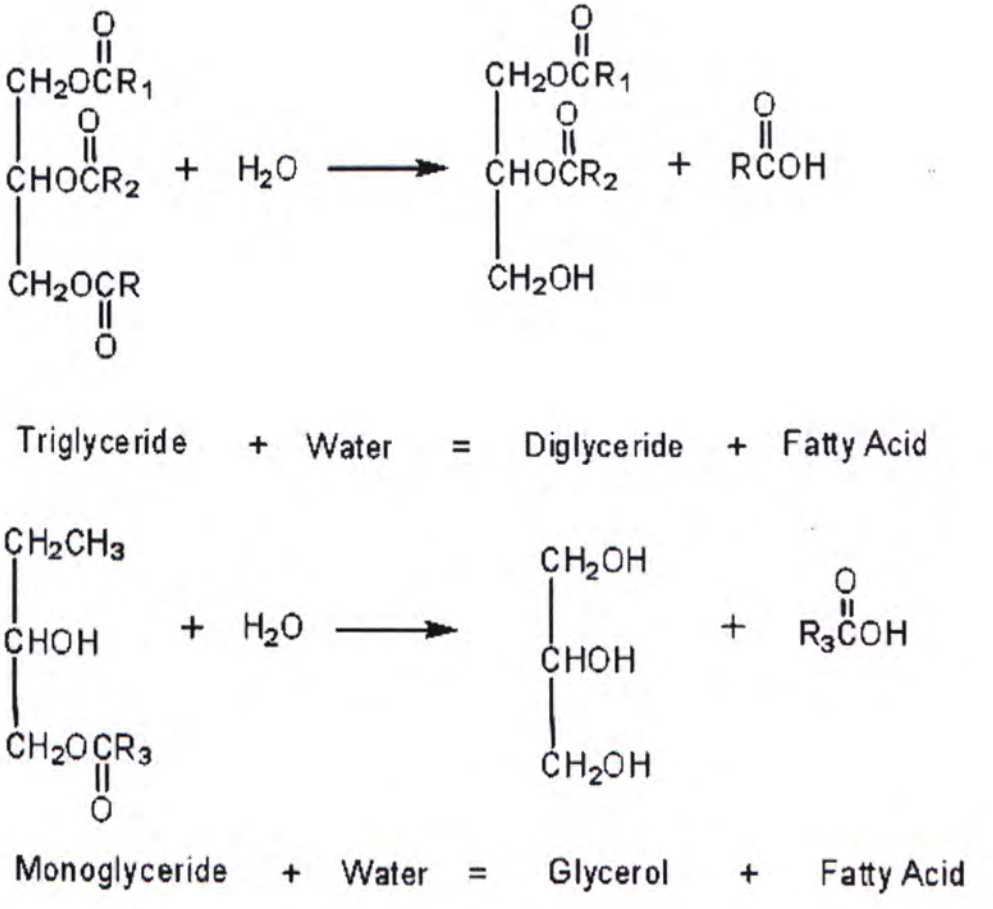


Figure 1.2 Hydrolysis process for frying oils. Modified from Gupta (Gupta, 2004).

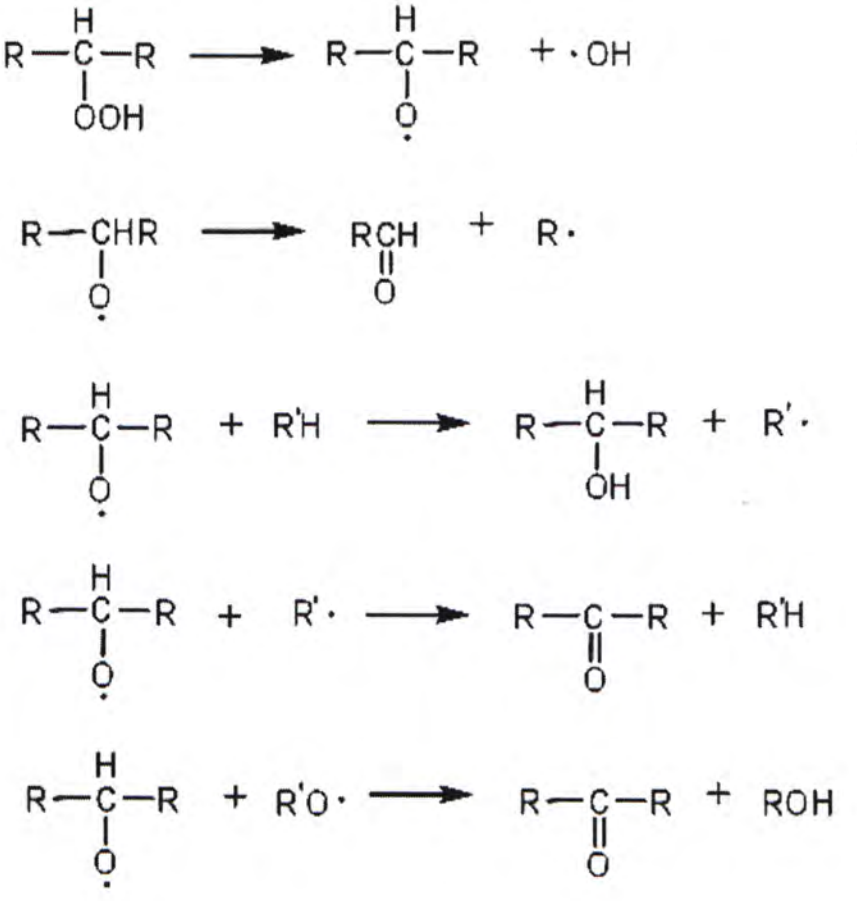


Figure 1.3 Oxidation process for frying oils. Adapted from Gupta (Gupta, 2004).

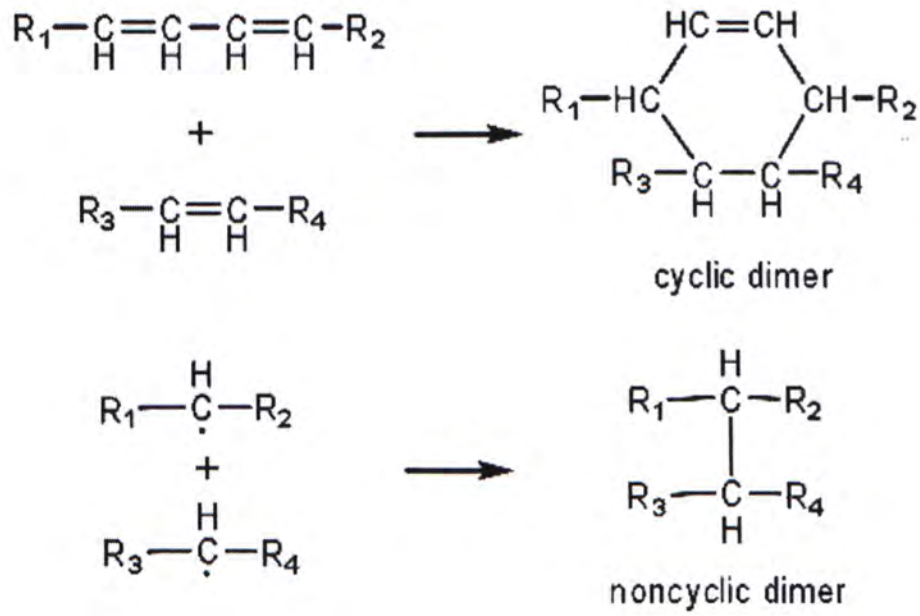


Figure 1.4 Polymerization process for frying oils. Modified from Gupta (Gupta, 2004).

1.1.3 Frying oil selection

Considering the six criteria in frying products – appearance, aroma/flavor, texture, mouth-feel, after-taste, and product shelf life (Gupta, 2004), the oils used in frying must have high oxidative and thermal stability with relatively lower price in the mean time. In addition, nutritional concerns have been on the top of the list of all progressive-minded snack food companies. Most well-known healthy frying oils are low in saturated fat and low in linolenic acid. Examples of such oils are high-oleic sunflower oil, high-oleic safflower oil and low-linolenic canola oil. However, the limited production of these healthy oils each year can not meet the need of current demand for frying oils in the food industrial. Hence traditional frying oils with reasonable price and sufficient supply, such as cottonseed oil, palmolein oil and partially hydrogenated soybean oil, are still popular in frying food manufacturing.

1.1.4 Quality control of frying oil

It has been mentioned above that the oil that is used for frying dishes in restaurants is usually determined by employees. Physical and chemical tests can be run to make a quick determination of oil quality. It can be either non-instrumental, including oil color, clarity and foam height observation, or through certain materials, e.g. paper strips to test shortening content by 3M™ company of St. Paul, MN, U.S.A.

To guarantee the safety of frying oil as well as that of fry foods during 1990 and 2001, 53 countries were contacted about regulations for frying fats and fried food, among which 34 countries have responded. Austria, Belgium, Chile, France,

Hungary, Italy and Spain have specific laws, regulations towards frying fats. While others don't except that Finland, Germany, the Netherlands, Norway Portugal and Sweden enforced measures for practical control in food establishment. State and local agencies in the United States are more concerned about the control and elimination of food-born pathogens (Firestone, 1993; Firestone *et al.*, 1991). In China, regional rules towards the control of fry foods and oils are more focused on the elimination of illegal additives such as aluminum, which leads to dementia. However, in the National Hygiene Standards for Food Additive Applications announced early this year, there is still no specific maxim restriction to food additives like aluminum potassium sulfate and aluminum ammonium sulfate (2008). And the prevention from using hogwash oils which contain substantial amount of hazardous compounds is also encouraged. Due to the early news on oil abuse in KFC in Mainland China, misuse of magnesium trisilicate to extend the frying life of oil in fast food restaurants became a public concern as well.

1.2 Selection of experiment oil

1.2.1 Lard as a cholesterol- containing animal fat

The fat tissue of pig that is not a part of the carcass or is trimmed off from it in preparing the carcass for market is the raw material from which lard is rendered. For many generations, lard was the fat of choice for preparing doughs and batters because of its plasticity at room temperature, which allows it to cream and aerate with sugar and egg yolk.

For cooking, lard has been treasured for centuries among Northern Europeans and Chinese. Today, the largest exporter and importer of the world is still European countries, followed by North America and the Caribbean and Asia (Parker, 2004).

The composition and physical characteristics of lard have wide variations due to the animal's diet and species. Moreover, hogs are monogastric and their stored fats closely resemble dietary intake; consequently, the fatty acid composition of pig fat is more closely related to the amount and fatty acid composition of the oils in its diet (Howe *et al.*, 2002; Myer *et al.*, 1992; Sandstrom *et al.*, 2000).

The structure of lard contains a high percentage of medium-melting, disaturated, monounsaturated triglycerides. These triglycerides are largely in a symmetrical arrangement, which cause lard to crystallize in β form. This characteristic has restricted the use of lard to applications requiring low structural properties but high lubricity. The function of lubricity is to impart tenderness and richness while improving eating quality by providing a feeling of satiety after eating

(O'Brien, 2004).

Major applications for lard due to the lubricity functionality are pie-crusts, yeast-raised doughs, and frying.

1.2.2 Corn oil as a healthy vegetable oil

Corn oil, also called maize oil, is usually a by-product in starch manufacturing. The corn germ is rich in oil (>30%), and is the source of all available commercial corn oil (Moreau, 2002). To be specific, it should be more accurately called corn germ oil.

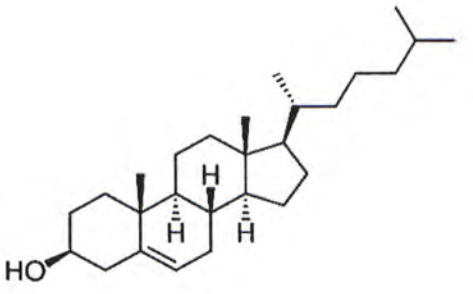
Between 1950 and 1970, a marketing slogan for corn oil was that it was "high in polyunsaturateds", mostly attributed to its high content of the essential fatty acid linoleic acid (18:2). Another desirable feature of corn germ oil is that it contains relatively low levels of saturated fatty acids and very low levels of linolenic acid (18:3). As earlier described, the low content of the linolenic acid makes the oil especially susceptible to oxidation. According to Stockwell, the more double bonds in a fatty acid, the more unstable the oil is and the more likely it is to undergo various forms of degradation, which makes the polyunsaturated oil less stable (Moreira, 1999a).

Additionally, the commercial corn oil has been recognized as containing the highest level of unsaponifiables (1.3~2.3%) of all the commercial vegetable oils (Strecker et al., 1996). The three main chemical components in the unsaponifiable fraction of corn oil are phytosterols, tocopherols and squalene (Moreau, 2002).

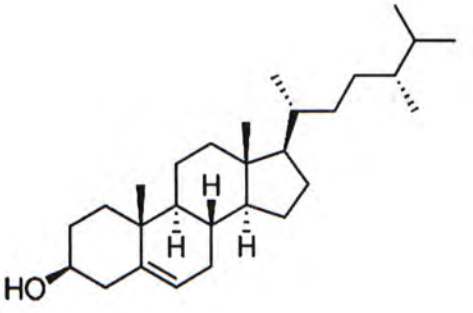
Phytosterols have been recognized as one of the twelve most important classes of phytonutrients (Fahey *et al.*, 1999) and a variety of food products enriched with plant sterols and stanols are on the market in many countries. The major phytosterols in corn oil are β-sitosterol > campesterol > stigmasterol (structures displayed in **Figure 1.5**), all of which are structurally related to cholesterol with a different side chain configuration at carbon C-24 (Ling *et al.*, 1995). Stanols are saturated sterols, which means that they have no double bonds in the sterol ring. Stanols are less abundant in nature (Salen *et al.*, 1985). Although cholesterol and phytosterols are similar in structure, the subtle difference on the C-24 side chain results in metabolism difference. Increasing complexity of the side chain increased hydrophobicity, which reduces absorption. While mammals do not synthesize phytosterols (Salen *et al.*, 1970), a research done by Heinemann et al. (Heinemann *et al.*, 1993) showed that the intestinal absorption of cholesterol, campestanol, campesterol, stigmasterol and sitosterol were 33,13,10, 5 and 4%, respectively in healthy men.

Maize oil has for a long time been considered a kind of superior frying oil for domestic purposes. This may due in part to its high sterol content which could impart a lower viscosity and thus better drainage from the fried food. However, since the corn oil is a by-product of the starch production, it has been considered relatively more expensive than other vegetable oils and seldom applied to industrial frying. Numerous studies have compared the stability of corn oil and other vegetable oils during frying (Gertz *et al.*, 2000; Strecker *et al.*, 1996). One frying study demonstrated that, compared to canola and soybean oils, corn oil produced the lowest level of oxidation products and retained the highest level of tocopherals

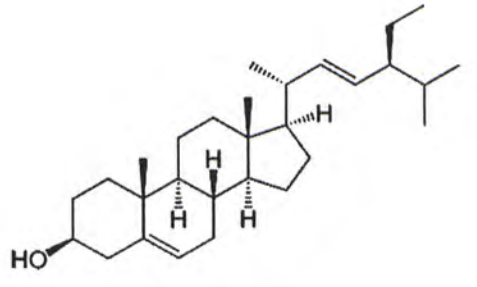
during 5 days at continuous frying temperatures (Strecker *et al.*, 1990).



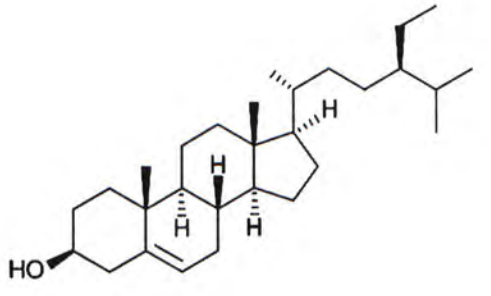
Cholesterol



Campesterol



Stigmasterol



β-Sitosterol

Figure 1.5 Structure of cholesterol and common phytosterols in corn oil

1.3 Current studies on frying oils

Researches using frying oil or thermally oxidized oil have tried to explore the possible effects of ingesting such oil on animals into various research fields. Rats, pigs, rabbits and transgenetic mice have been used as animal models to study mechanisms on how the oxidized frying oil affects lipid compositions, hormone level, oxidative stress and cholesterol metabolism (Battino *et al.*, 2002; Eder, 1999; Eder *et al.*, 2000; Garrido-Polonio *et al.*, 2004; Khan-Merchant *et al.*, 2002; Liu *et al.*, 1995; Liu *et al.*, 1996; Martin *et al.*, 2000; Narasimhamurthy *et al.*, 1999a; Narasimhamurthy *et al.*, 1999b; Rong *et al.*, 2002; Staprans *et al.*, 2005).

Studies referring on the effects of prolong-heated or fried oil on cholesterol level have been carried out *in vitro*, the results were contradictory and the underlying mechanism has not been elucidated (Battino *et al.*, 2002; Eder, 1999; Garrido-Polonio *et al.*, 2004). Recently, Koch K *et al.* found out that by feeding thermally oxidized oil to rats, a reduction of the cholesterol content in plasma and liver occurred with increased expression of insulin-induced genes and inhibited activation of sterol regulatory element-binding protein in rat liver, which in turn inhibited transcription of proteins involved in hepatic cholesterol synthesis and uptake (Koch *et al.*, 2007). Together with other related researches, such mechanism was supported (Chao *et al.*, 2001; Konig *et al.*, 2007; Luci *et al.*, 2007; Sulzle *et al.*, 2004).

1.4 Atherosclerosis and cholesterol metabolism

1.4.1 Atherosclerosis

Atherosclerosis, still the leading cause of morbidity and mortality in the western world (Braunwald, 1997), is the pathological process underlying a group of syndromes that can be divided into 3 main categries: (1) coronary heart disease (CHD), (2) cerebrovascular disease, like stroke (Posner *et al.*, 1991) and (3) peripheral artery disease. The development of an atherosclerotic lesion is usually involved with 3 stages: initiation, progression, and regression. It is usually developed in the arterial intima where vascular smooth muscle cells (VSMCs), inflammatory cells, lipids and connective tissue accumulate. Initiation of lesion progression occurs due to dysfunction of the endothelium resulted from insudation of lipids into the vessel wall which results in a monocytes rolling and then adhering to the endothelium prior to migrating into the intima, thus caused a macrophage collection. The macrophages ingest lipid to become resident foam cells mainly through accumulation of low-density lipoprotein (LDL). In this process, highly oxidized LDL was shown be a stimulating factor (Dwivedi *et al.*, 2001; Frostegard *et al.*, 1992; Rosklint *et al.*, 2002). A fibrous cap which separates the lipid core and the lumen is created through VSMCs migration and proliferation over the lipids, while the macrophages remain releasing matrix metalloproteinases which would lead to final rupture of the cap. The platelets accumulate later at the site followed by thtombus formation, after which, with VSMCs migrating into the thrombus, depositing new matrix and cells, a new fibrous cap is created. In this way, the atherosclerotic plaque grows continuously by repeated rupture and repair cycle (**Figure 1.6**).

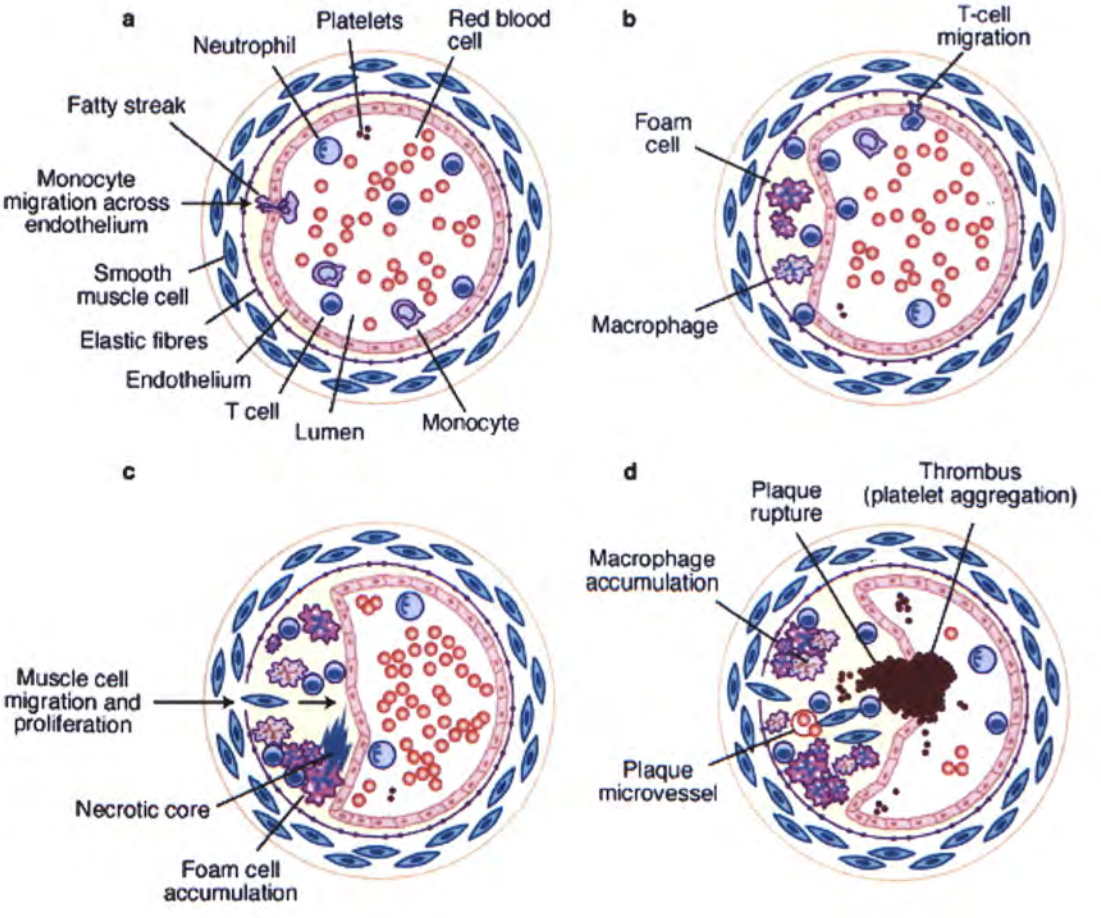


Figure1.6 Stages of atherosclerotic plaque development. Adapted from Stevens et al. (Stevens *et al.*, 2005).

Atherosclerotic lesions can occur as early as adolescence and progression of disease relies on genetic and environmental factors (Lusis, 2000). It involves the thickening and hardening of arteries, and usually affects large and medium-sized arteries. There can be a single blockage or multiple blockages, and they vary in severity and location (**Figure 1.7**). Any narrowing, or blockage, of the coronary arteries reduces the blood supply to the heart tissue, therefore reducing the amount of oxygen and nutrients delivered. This inhibits the normal function of the heart muscle. Due to the highly complex nature of atherosclerosis, the molecular and cellular interactions mediating disease progression are not completely understood.

The effect of fatty acids on plasma lipoproteins has been studied extensively (Ginsberg *et al.*, 1998; Mensink, Zock *et al.*, 1992; Yu *et al.*, 1995). SFAs increase total cholesterol and LDL cholesterol (LDL-C) (Mensink *et al.*, 1992), whereas monounsaturated fatty acids and PUFAs decrease LDL-C (Kris-Etherton *et al.*, 1997). A number of epidemiologic studies have shown a clear association between dietary saturated fat and atherosclerosis (Posner *et al.*, 1991). Dietary cholesterol has also been shown to raise total and LDL cholesterol; however, its direct association with atherosclerosis is rather weak (Hegsted *et al.*, 1993).

The HDL cholesterol has been paid a lot of attention for the past decades due to its protective role in prevention of atherosclerosis (Santos-Gallego *et al.*, 2008). Drugs, food, physical exercise, smoking cessation, weight control and moderate alcohol intakes have shown to increase HDL cholesterol levels, which is also applicable to reduce certain risk factors for atherosclerosis and CVD (Langslet *et al.*, 2008; Nomiyama *et al.*, 2008).

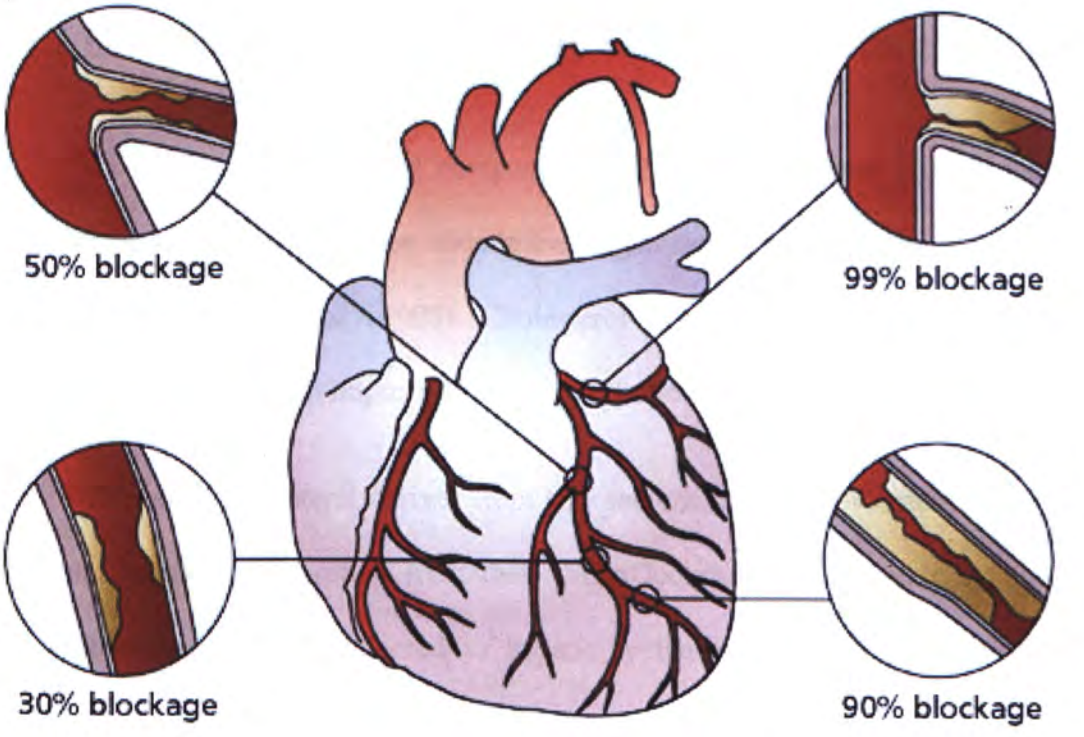


Figure 1.7 Different severity and locations of artery blockage of human heart. Adapted from Richer et al. (Richer *et al.*, 1999).

1.4.2 Cholesterol metabolism and related regulating factors

As an essential structural component of cellular membrane in mammalian cells, cholesterol can influence membrane organization and thereby membrane properties (Maxfield *et al.*, 2005). Cholesterol is also the precursor of steroid hormones, which plays an important role in metabolic control.

The body cholesterol derives from two sources, i.e., *de novo* biosynthesis in liver and intestinal absorption from diet. When looking at whole-body synthesis, much attention has been devoted to hepatic synthesis (Mazier *et al.*, 1997). It accounts for 40-50% of whole-body newly synthesized cholesterol in selected primates, 70% in baboons and 10% in cynomolgus monkeys (Dell *et al.*, 1985; Spady and Dietschy, 1983; Turley *et al.*, 1995). Cholesterol is synthesized from two-carbon acetyl-CoA moieties, the rate limiting enzyme in this pathway is HMG-CoA reductase, a highly regulated enzyme that catalyses the conversion of HMG-CoA to mevalonate. However, cholesterol itself also exerts a negative feedback on HMG-CoA reductase activity. When sterol levels are high in the endoplasmic reticulum (ER) membrane, the ebiquitination and degradation of HMG-CoA reductase is enhanced through binding to the sterol-sensing domain of insulin-induced genes (Insig)-1 ansd -2(Sever *et al.*, 2003; Sever *et al.*, 2003). Besides this, cholesterol can also regulate gene expression of HMG-CoA reductase by blocking the activation of the transcription factor, sterol regulatory element-binding protein-2 (SREBP-2). SREBP-2 is one of the three known SREBP isoforms: SREBP-1a, -1c and -2. All of them belong to a large class of transcriptional factors containing basic helix-loop-helix-Zip domains (Horton, 2002; McPherson *et al.*, 2004). After synthesis in the ER

membrane, SREBP binds to the SREBP cleavage-activating protein (SCAP), which is a 1276 amino acid polytopic membrane protein that escorts SREBP from the ER to the Golgi under sterol depletion situation (Brown *et al.*, 1997; Goldstein *et al.*, 2002; Horton *et al.*, 2002). In the Golgi, SREBP undergoes two sequential cleavages by Site-1 protease (S1P) and Site-2 protease (S2P) to generate its transcriptionally active NH_2 fragment, which can then translocate to the nucleus and activate transcription of target genes. The above-mentioned Insig-1 and -2 can also bind to SCAP when intracellular sterol concentrations increased. This action facilitates the retention of SCAP/SREBP complex in the ER, which will later result in the declined synthesis of cholesterol and fatty acids (Yang *et al.*, 2002). The mechanism in this pathway is still not fully understood.

SREBP-1a, -1c, and -2 play different but critical roles in both cholesterol homeostasis and lipogenesis. SREBP-1a and -1c are produced by the same gene with different first exons which spliced to a common second exon (Osborne, 2000). SREBP-1a is a potent transcriptional activator of all SREBP-responsive genes owing to the long transactivation domain encoded by exon 1a, but it is also constitutively expressed at much lower levels in liver than the other two forms (Shimomura *et al.*, 1997), which suggests its possible responsibility for maintaining basal levels of cholesterol and fatty acid synthesis *in vivo* (Horton, 2002; Shimomura *et al.*, 1997). Under normal situations, SREBP-1c preferentially activate genes involved in fatty acid synthesis, like ATP citrate lyase (producing acetyl-CoA), acetyl-CoA carboxylase (ACC) and fatty acid synthesis (FAS), SREBP-2 preferentially activates the LDL-receptor genes and various other genes required for cholesterol synthesis, such as HMG-CoA reductase (McPherson *et al.*, 2004). Besides, both SREBP-1c and

-2 regulate three genes, malic enzyme, glucose-6-phosphate dehydrohenase (G6PD) and 6-phosphogluconate dehydrogenase, which are required fro the generation of NADPH molecules used at multiple stages of both cholesterol and lipid biosynthetic pathways (Horton, 2002; Horton et al., 2002).

And in the intestine, after uptake by enterocytes, cholesterol is packed with triglycerides into chylomicrons (CMs) and secreted into lymph. While the TG are hydrolyzed and free fatty acids (FFA) are taken up by the peripheral tissues, cholesterol-enriched chylomicron remnants are rapidly taken up by the liver, which influences hepatic cholesterol synthesis. The liver can either dispose the excess cholesterol by esterification of cholesterol with acyl CoA cholesterol acyltransferase (ACAT) in cytoplasmic lipid droplets for temporary storage. Cholesteryl ester, the esterification product can later be hydrolyzed to liberate free cholesterol when necessary. Or the liver, like the intestine, can secret very low density lipoprotein (VLDL) particles, which consist of a neutral lipid core composed of cholesteryl esters and triacylglycerols and a monolayer surface containing phospholipids, free cholesterol, and a variety of apolipoproteins.

Finally, the cholesterol could be converted into bile acids by hepatocytes, followed by their secretion into the bile along with significant amounts of neutral sterols. The main bile acids synthetic pathway, with cholesterol 7α-hydroxyase (CYP7A1) as its rate-limiting enzyme, is under feedback regulation. Farnesoid X receptor (FXR), a bile acid receptor that binds to the bile acid response element of the genes coding for several enzymes in the bile acid synthetic pathways can thus prevent a transactivating factor from binding to the site, inhibiting CYP7A1

transcription. Another nuclear receptor, the liver X receptor (LXR), with oxysterols as its ligands (Chiang, 2002; Edwards *et al.*, 2002) can activate CYP7A1 transcription by binding to the LXR response element present in the promoter region of the CYP7A1 gene. Therefore, regulation of CYP7A1 and bile acid synthesis depends on the balance of positive LXR control and negative FXR control. In addition, LXR activates a variety of genes involved in cholesterol metabolism, including genes encoding ATP-binding cassette transporters and some proteins in reverse cholesterol transport.

Peripheral cells, such as muscle, fat cells, are not able to form lipoprotein or to metabolize cholesterol extensively. Therefore, these cell types depend massively on efflux pathways for removal of their excess cholesterol. It is generally accepted that high-density lipoprotein (HDL) is the primary acceptor for cholesterol efflux from cells. HDL cholesterol can be taken up by the liver for further processing. The pathway is generally referred to as the reverse cholesterol transport (RCT) pathway (Barter, 2002). The RCT pathway is particularly important in the removal of excess cholesterol from other tissues. A common hypothesis is that high HDL cholesterol levels decrease the risk for CVD by removing the excess of cholesterol from the macrophages and enhancing RCT. However, recent work implied that the above-mentioned concepts that the liver plays an important role in the RCT need re-definition considering the contribution of intestine in cholesterol homeostasis (Groen *et al.*, 2004).

The general view of the involvement of liver and intestine in cholesterol metabolism is illustrated in **Figure 1.7**.

Besides the above-mentioned LXR and FXR, there are other nuclear receptors shown to be involved in the regulation of plasma lipoprotein metabolism, which are the estrogen receptors (ERs) and the fatty acid receptors – peroxisome proliferator activated receptors (PPARs). The PPAR family includes three members encoded by distinct genes: α, β/δ, and γ (Kersten *et al.*, 2000). After the initial discovery of PPARα isotype in 1990 (Issemann *et al.*, 1990), a large amount of literature on these receptors has accumulated. It was shown recently that dietary oxidized fats (Chao *et al.*, 2001) as well as cyclic fatty acid monomers (Martin *et al.*, 2000), components of heated fats, lead to activation of the PPARα and cause an increased expression of its target genes and thus lead to alternations in cholesterol levels. It will be stated in detail in the discussion part of this thesis.

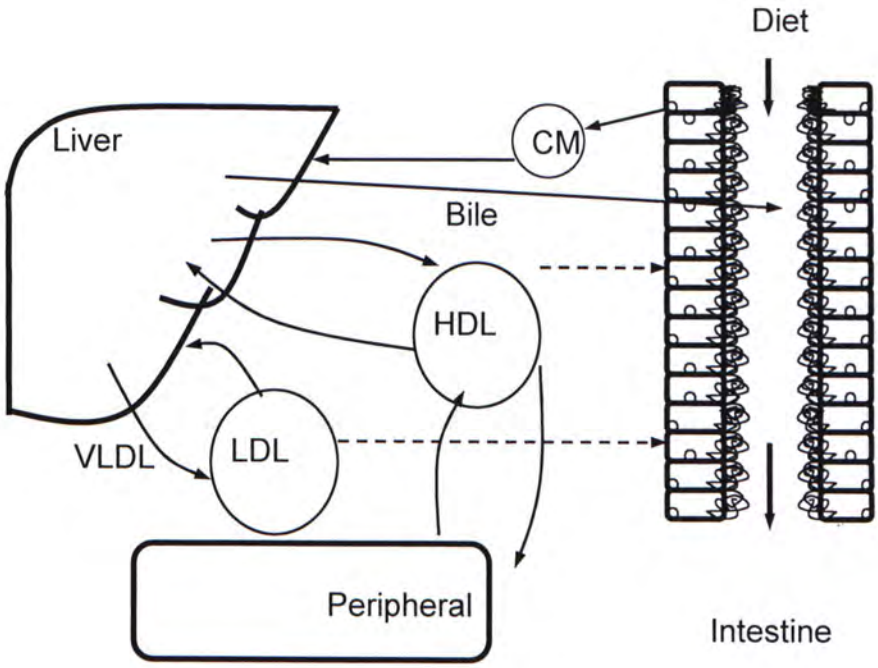


Figure 1.7 Overview of the involvement of intestine and liver in cholesterol homeostasis.

1.5 Animal model selection

The research for effective lipid-lowering drugs and nutraceuticals is usually based upon an assessment of their effects on circulating lipids in laboratory animals. Although the rat is the most commonly used species for the detection and evaluation of agents affecting lipid metabolism, the difference between the distribution of lipids in rat and human serum lipoproteins can not be ignored (Dvornik *et al.*, 1980). When compared to most other small experimental animals, lipid lipoprotein metabolism in hamsters is comparable to that in human because of similar component and metabolism of both lipoproteins and bile acids (Bravo, D. A., Gleason *et al.*, 1994; Bravo, E., Cantafora *et al.*, 1994; Plancke *et al.*, 1988; Singhal *et al.*, 1983; Spady, Bilheimer *et al.*, 1983; Spady and Dietschy, 1983; Suckling *et al.*, 1991; Weingand *et al.*, 1991).

Chapter 2

Objectives

The aim of this study was to investigate whether and how the heat-abused oil influenced the cholesterol metabolism in hamsters. Commercial lard and corn germ oil were chosen because of their compatibility to frying due to different features, and as references of both animal fats and vegetable oil. So far, no such experiments have been done on hamster, which is an appropriate model to explore the plasma lipid metabolism.

Chapter 3

Materials and Methods

3.1 Sample lard and corn oil preparation

Refined lard (15kg, BHA 200 PPM max, Evergreen Oil & Fats Ltd. Co. Hong Kong) and commercial corn oil (1 gallon Mazola® corn oil, cholesterol and BHT/ BHA free, Unilever Corp. Hong Kong) which is sealed stored at room temperature (T<20 °C), were used for frying. A family deep fryer was purchased from Philips Corporation (Prod. No.: HD6159). Before frying, the oil container was washed with iron-free water and air-dried. Oil (1200 g) was weighed each time, heated at 180°C, 9 hours a day for continuously 2, 4, 6 days respectively. After the heating process was completed, it was filtered when the oil cooled down to about 80 °C. Oil sample (1000 g) was weighed and stored in an air-tight box in -20°C fridge until diet preparation. The rest of the heated lard was flushed with nitrogen and sealed stored in separated test tubes and micro tubes in -20°C fridge until analysis. During all frying processes, the temperature was monitored by a calibrated thermometer. The color of fresh and fried lard /corn oil samples under frozen state is shown in Figure 3.1 and Figure 3.2.

Figure 3.1 Color of non-fried and fried lard samples

Samples from left to right are fresh lard, lard fried for 2d, 4d, and 6d.

Figure 3.2 Color of non-fried and fried corn oil samples

Samples from left to right are fresh corn oil, corn oil fried for 2d, 4d, and 6d.

3.2 Diet preparation

The control diet was prepared by mixing the following ingredients in proportion (g/kg diet): cornstarch, 568; casein, 200; lard, 100; sucrose, 50; mineral mix AIN-76, 40; vitamin mix AIN-76A, 20; DL-methionine, 1; cholesterol, 1. In the experiment that used lard, the three previously stored fried lard products were melted at 60 °C and added into powdered diet mix respectively to make the three experimental diets while in corn oil experiment, the stored corn oil samples were thawed at room temperature before mixing. The blended diets were then mixed with a gelatin solution (50g/L) in a ratio of 2.5kg diet per liter of solution. Once the gelatin was set, the diets were quickly divided into pieces of approximately 20 g cubes, air drying overnight and stored frozen at -20°C. Diet composition is shown in **Table 3.1**.

Table 3.1 *Composition of the diets for 8 groups of hamsters (g/kg diet)*

	NFL	2FL	4FL	6FL	NCO	2FCO	4FCO	6FCO
Corn starch	568	568	568	568	568	568	568	568
Casein	200	200	200	200	200	200	200	200
Sucrose	50	50	50	50	50	50	50	50
Mineral mix (AIN-76)	40	40	40	40	40	40	40	40
Vitamin mix (AIN-76A)	20	20	20	20	20	20	20	20
DL-methionine	1	1	1	1	1	1	1	1
Cholesterol	1	1	1	1	1	1	1	1
Fresh Lard	**100**	0	0	0	0	0	0	0
Fried 2d Lard	0	**100**	0	0	0	0	0	0
Fired 4d Lard	0	0	**100**	0	0	0	0	0
Fried 6d Lard	0	0	0	**100**	0	0	0	0
Fresh Corn Oil	0	0	0	0	**100**	0	0	0
Fried 2d Corn Oil	0	0	0	0	0	**100**	0	0
Fired 4d Corn Oil	0	0	0	0	0	0	**100**	0
Fried 6d Corn Oil	0	0	0	0	0	0	0	**100**
Gelatin	20	20	20	20	20	20	20	20

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days. NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

3.3 Animals

In each experiment, 45 male adult Golden Syrian hamsters (*Mesocricetus auratus*, the Laboratory Animal Services Centre, the Chinese University of Hong Kong) were randomly assigned to 15 cages of 3 hamsters each, with an initial weight of 106 ± 4 (SD) g for lard experiment and 110 ± 6 (SD) g for corn oil experiment. After 1 week of acclimation to the wire-bottomed cage on animal chow diet, they went on a 2-week stabilization period fed on a high fat high cholesterol diet (control diet). After the stabilization, the hamsters were reassigned to 4 groups (n=11~12 each) and maintained on four experimental diets for 7 weeks. Accordingly, the experiment groups were named after the oil type that consumed as non-fried lard (NFL), fried lard for 2 days (2FL), for 4 days (4FL), and for 6 days (6FL) in lard experiment and non-fried corn oil (NCO), fried corn oil for 2 days (2FCO), for 4 days (4FCO), and for 6 days (6FCO) in corn oil experiment. During the study, the hamsters were housed in an environmental controlled atmosphere (temperature, 23°C; light, 12-hour-light/-dark cycle) with free access to food and tap water. The health status of the hamsters was monitored daily, experimental diets were given daily and uneaten food was discarded. Body weights were recorded every week.

3.4 Sample collection

Blood was sampled from the retro-orbital sinus by a capillary tube into a heparinized microtube at the end of week 0, 3 and 6 after a 16- to 18-hour fasting. The blood was centrifuged at 3000 rpm for 10 min at 4 °C and the plasma was obtained and stored at -20°C until analysis.

After the blood collection at the end of week 6, the hamsters were kept for one more week for recovery. After food deprivation for 16 to 18 hours, the hamsters were asphyxiated with nitrogen. And blood was collected from the abdominal aorta into a vacuum heparinized tube, centrifuged at 2,500 rpm for 10 min at 4 °C, and the plasma was prepared and stored at - 20°C until analysis. Liver, heart, kidney, perirenal adipose tissue and epididymal adipose tissue were removed, washed in saline, weighed, snap- frozen in liquid nitrogen and stored at -80°C until analysis.

Hamster fecal samples were collected at the end of week 1 and week 6 for lard experiment and fecal sample of week 3 was also collected in corn oil experiment.

3.5 GC analysis of fatty acid composition in fresh and fried experiment oil samples

To analyze the fatty acid composition of the oil samples, 1 mg triheptadecanoate (internal standard) was added to c.a. 10 mg of oil sample in hexane in separate tubes, which were then dried under a gentle stream of nitrogen. 2 mL of 14% boron trifluoride (BF_3) in methanol and 1 mL of toluene were added to methylate the samples. All tubes were flushed with nitrogen for a few seconds before capped. The samples were mixed and heated at 90°C for 1 hour. After that, samples were extracted by 3 mL of hexane and 1 mL of distilled water. Organic phase was obtained by centrifugation at 2,000 rpm for 10 min. The samples were then dried under nitrogen and 0.8 ml hexane was added. These samples were later transferred to GC vials subjected to GC analysis.

The fatty acid methyl esters were analyzed on a flexible silica capillary column (Innowax 19091N-213, 30m x 0.32mm i.d., Alltech, Inc., USA) in a Shimadzu GC 2010 gas chromatograph equipped with a flame-ionization detector (Palo Alto, CA, USA). Column temperature was programmed from 150 to 200°C at a rate of 15°C/min and then gradually increased from 200 to 250°C at the rate of 2°C/min, after which was maintained at 250°C for 5 min. Injector and detector temperature were set at 220°C and 270°C respectively. Helium was used as the carrier gas at a head pressure of 15 psi. Identification of each fatty acid methyl ester was made by comparison of retention time of authentic standards (Sigma Chemical Co., St. Louis, MO, USA). Fatty acids were quantified based their peak areas. Typical chromatogram of fatty acids in corn oil is shown in **Figure 3.3** and **Figure 3.4**

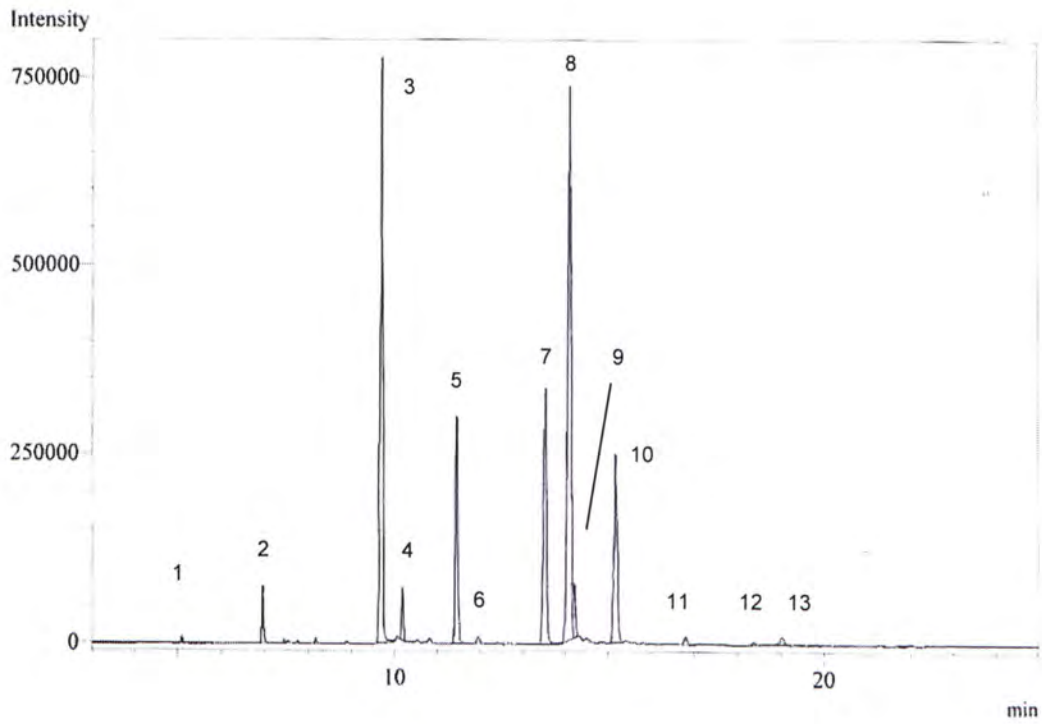


Figure 3.3

Gas liquid chromatogram of fatty acids of lard. Identification of peaks:

1, 12:0; **2**, 14:0; **3**, 16:0; **4**, 16:1(n-9); **5**, 17:0(Internal Standard); **6**, 17:1; **7**, 18:0; **8**, 18:1(n-9); **9**, 18:1(n-7); **10**, 18:2(n-3); **11**, 18:3(n-3); **12**, 20:0; **13**, 20:1(n-11);

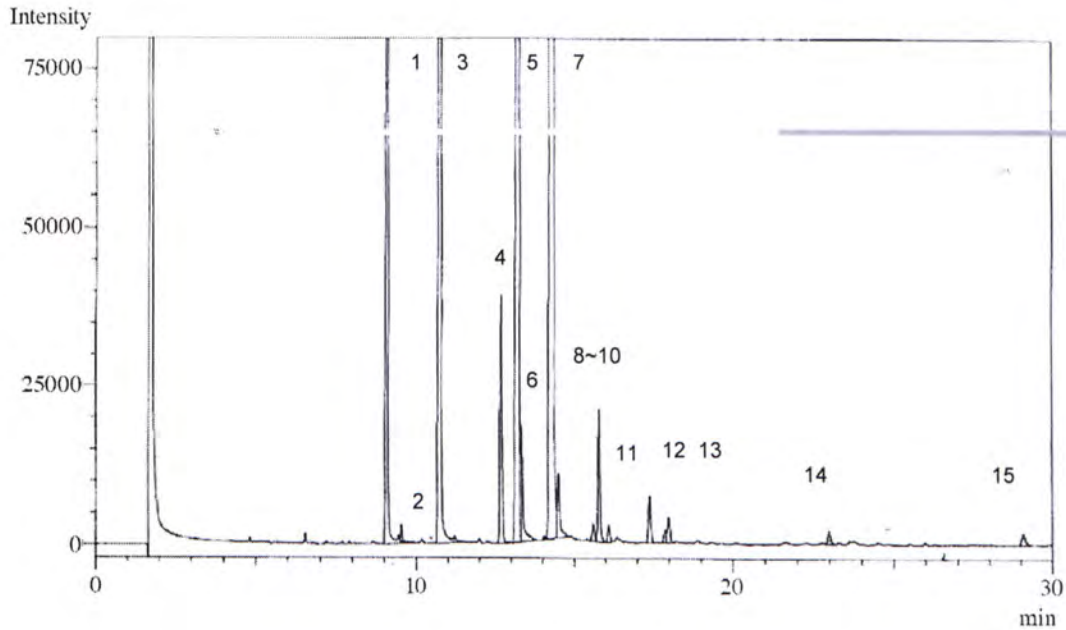


Figure 3.4

Gas liquid chromatogram of fatty acids of corn oil. Identification of peaks:

1. 16:0; **2**. 16:1; **3**. 17:0(Internal Standard); **4**. 18:0; **5**. 18:1(n-9); **6**.18:1(n-7); **7**.18:2(n-3); **8~10**.18:3(n-3); **11**. 20:0; **12**. 20:1(n-9); **13**. 20:1(n-7); **14**. 22:0; **15**. 24:0;

3.6 Determination of plasma cholesterol and organ cholesterol

Plasma total cholesterol (TC) and triglyceride (TG) were measured by commercial enzymatic kits from Infinity (Waltham, MA, USA) and Stanbio Laboratories (Boerne, TX, USA) respectively according to the manufacturers' instructions. For measurement of plasma HDL-cholesterol, LDL and VLDL were first precipitated with phosphotungstic acid and magnesium chloride in a commercial kit (Stanbio) and HDL-cholesterol in the supernatant was determined similarly as processing TC. Non-HDL cholesterol was calculated by deducting HDL-cholesterol from TC.

Cholesterol contents in the hamster liver and kidney were determined by a method described by (Chan et al., 1999). Briefly, 1 mg of stigmastanol (internal standard) was added into about 100 mg of liver sample (c.a. 300 mg of kidney sample). 15 mL of methanol-chloroform mixture (2:1, vol/vol) were used to extract lipids from the tissue together with 3 mL of saline, and the chloroform-methanol phase was saved and evaporated to dryness under a nitrogen stream. The lipids were then mildly saponified with 5 mL of 1N NaOH in 90% ethanol at 90°C for 1 hour, followed by the extraction of non-saponified substances (including cholesterol) by 6mL of cyclohexane together with 1 mL of water. The extracted substances were then converted into their trimethylsilyl-ether derivatives by a commercial trimethylsilyl reagent (dry pyridine-hexamethyldisilazane, 9: 3: 1, vol/vol/vol, Sil-A Reagent, Sigma) at 60°C for 1 hour. After drying up under nitrogen, the cholesterol

trimethylsilyl-derivatives dissolved in 600 μL of n-hexane and transferred into GC-vials and were analyzed in a fused silica capillary column (SAC™-5, 30m x 0.25mm internal diameter, Supelco, Inc., Bellefonte, PA, USA) in a Shimadzu GC-14B GLC equipped with a flame ionization detector (Shimadzu, Tokyo, Japan). The column temperature was set at 285°C and held for 25 minutes. Injector temperature and detector temperature were both set at 300°C. Helium was used as the carrier gas at a head pressure of 150 kPa. Amount of cholesterol in the tissue was calculated as a ratio of internal standard added. Typical chromatogram of cholesterol is shown in **Figure 3.5**.

Cholesterol contents in lard were determined with the method described above using c.a. 300 mg lard sample.

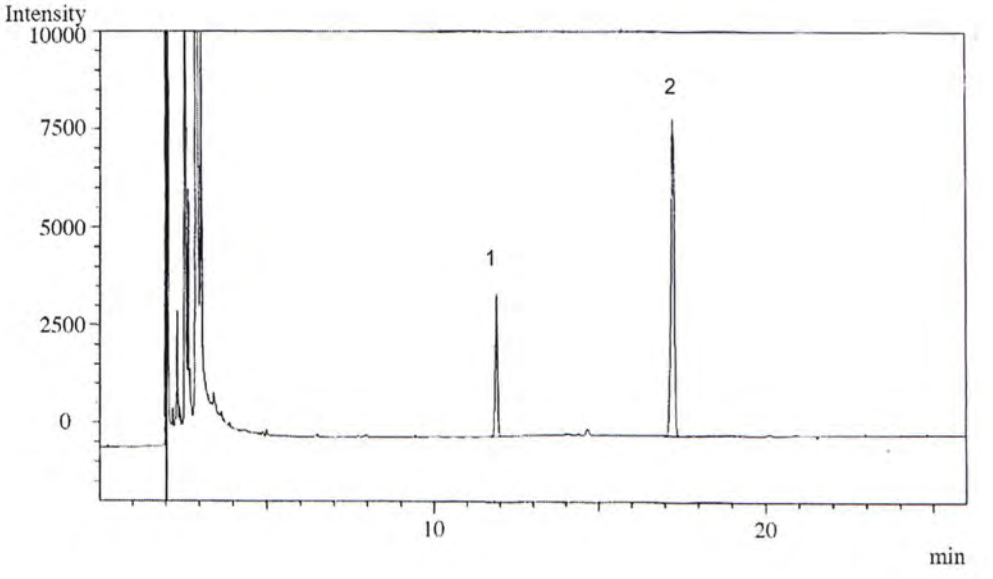


Figure 3.5

Gas liquid chromatogram of organ cholesterol. Identification of peaks:

1, Cholesterol; **2**, Stigmastanol (Internal Standard).

3.7 Determination of hamster fecal neutral and acidic sterols, corn oil phytosterol content

Neutral and acidic sterols in the feces of the hamsters were determined by a method described by Czubayko et al. (Czubayko et al., 1991) with slight modifications. The feces collected were first isolated and dried in lyophilizer, weighed and ground into powder by a coffee bean mill. 0.5 mg of stigmasterol (Sigma Chemical Co., St. Louis, MO, USA) in 200 μL of chloroform was added into a methylation tube as internal standard for neutral sterols and dried under a gentle stream of nitrogen. About 300 mg of lyophilized fecal sample was added into a methylation tube together with 0.5 mg hyodeoxycholic acid in 200 mL 1N NaOH in 90% EtOH as internal standard for acidic sterols. The samples were then mildly hydrolyzed with 8 mL 1N NaOH in 90% ethanol at 90°C for 1 hour. Then, 8 mL of cyclohexane together with 1 mL of distill water were added for the extraction of total neutral sterols. The methylation tubes were centrifuged to separate the neutral sterol-containing upper cyclohexane phase and the acidic sterol-containing lower aqueous phase. The two phases were separately analyzed as described below.

3.7.1 Determination of fecal neutral sterols

The cyclohexane phase was transferred into a new methylation tube and was evaporated under a gentle nitrogen stream. The sterols were converted into their trimethylsilyl derivatives using a commercial trimethylsilyl reagent (dry pyridine-hexamethyldisilazane, 9: 3: 1, vol/vol/vol, Sil-A Reagent, Sigma Chemical Co., St. Louis, MO, USA) at 60°C for 1 hour. After dried by nitrogen stream, the sterol

derivatives were dissolved in 400 μL of n-hexane and transferred into a GC vial and were analyzed in a fused silica capillary column (SAC™-5, 30 m x 0.25 mm internal diameter, Supelco) in a Shimadzu GC-14B GC equipped with a flame-ionization detector (Shimadzu). Helium was used as the carrier gas at head pressure 150 kPa and the column temperature was maintained at 280°C for 30 minutes. Injector and detector temperature were set at 300°C respectively. Retention time of each neutral sterol (coprostanol, coprostanone, cholesterol and dihydrocholesterol) was compared with that of authentic standards (Sigma Chemical Co., St. Louis, MO, USA). A typical chromatogram of fecal neutral sterols of hamsters fed lard is shown in **Figure 3.6**.

Neutral sterols in the feces of the hamsters that fed corn oil were determined exactly as described above except for the replacement of internal standard from stigmasterol to 5α-cholestane due to the presence of phytosterols, including stigmasterol. A typical fecal neutral sterol chromatogram of hamsters fed corn oil is shown in **Figure 3.7**.

3.7.2 Determination of fecal acidic sterols

The bottom aqueous layer described in 3.7.1 was saved for determination of acidic sterol content. 1 mL of 10N NaOH was added and the mixture was heated at 120°C for 3 hours. 3 mL of distill water and 1 mL of 25% HCl were then added and the acidic sterols were extracted by 7 mL of diethyl ether twice. The ether phases were pooled and dried under a gentle stream of nitrogen. Then, 2 mL of methanol, 2 mL of 2,2-dimethoxypropane and 40μL of concentrated HCl were added and the mixture was allowed to stand overnight, followed by evaporation to dryness under

nitrogen. 300 μL of a commercial trimethylsilyl reagent (dry pyridine-hexamethyldisilazane, 9: 3: 1, vol/vol/vol, Sil-A Reagent, Sigma Chemical Co., St. Louis, MO, USA) was added and the acidic sterols were converted into their trimethylsilyl derivatives at 60°C for 1 hour. The solvent was dried up under a gentle nitrogen stream and dissolved in 300 μL of n-hexane. After centrifugation, the supernatant was transferred into a GC vial and analyzed by a fused silica capillary column (SAC™-5, 30 m x 0.25 mm internal diameter, Supelco) in a Shimadzu GC-14B GC equipped with a flame-ionization detector (Shimadzu). Helium was used as the carrier gas at head pressure 150 kPa and the programme was set from 230°C to 280°C at a rate of 1°C/ min. Injector and detector temperature were set at 260°C and 300°C respectively. Retention time of each acidic sterol (lithocholic acid, deoxycholic acid, chenodeoxycholic acid, cholic acid and ursodecholic acid) was compared with that of authentic standards (Sigma Chemical Co., St. Louis, MO, USA). A typical chromatogram is shown in **Figure 3.8**.

3.7.3 Determination of phytosterol content in corn oil.

The phytosterol content in experimental corn oil was determined by adding 1 mg 5α-cholestane to approximately 300 mg oil sample as an internal standard due to the presence of stigmasterol. The samples subjected to GC analysis were prepared followed the organ cholesterol determination method. The GLC detection method is the same as that is used to detect organ cholesterol. Peaks were identified with authentic standards purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). A typical chromatogram of phytosterols in corn oil is shown in **Figure 3.9**.

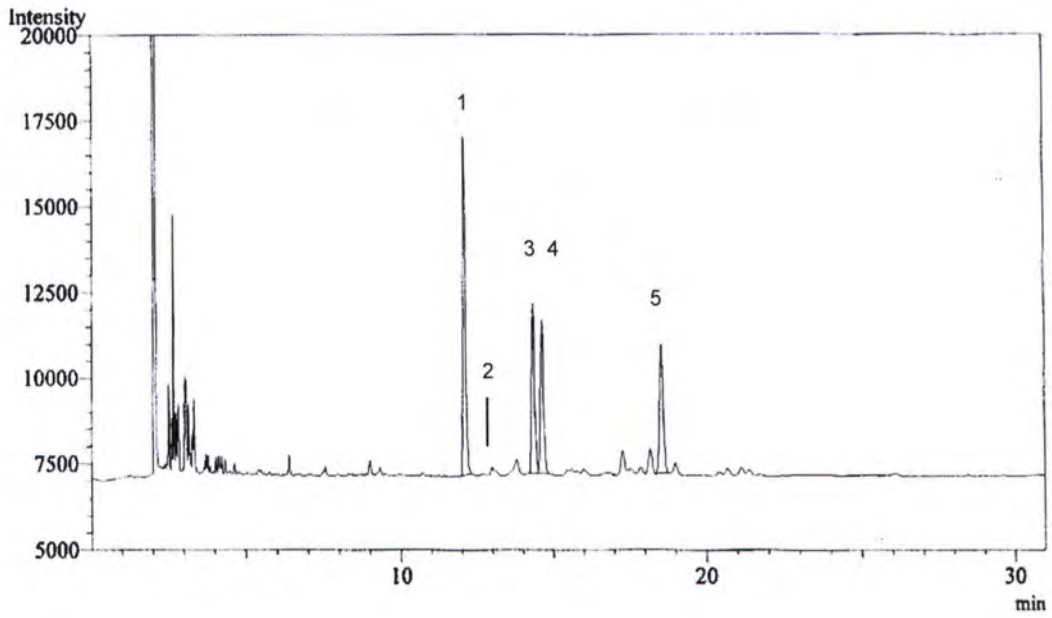


Figure 3.6

Gas liquid chromatogram of fecal neutral sterols of hamsters fed lard.

Identification of peaks:

1, Coprostanol; **2**, Coprostanone; **3**, Cholesterol; **4**, Dihydrocholesterol; **5**, Stigmasterol (Internal Standard);

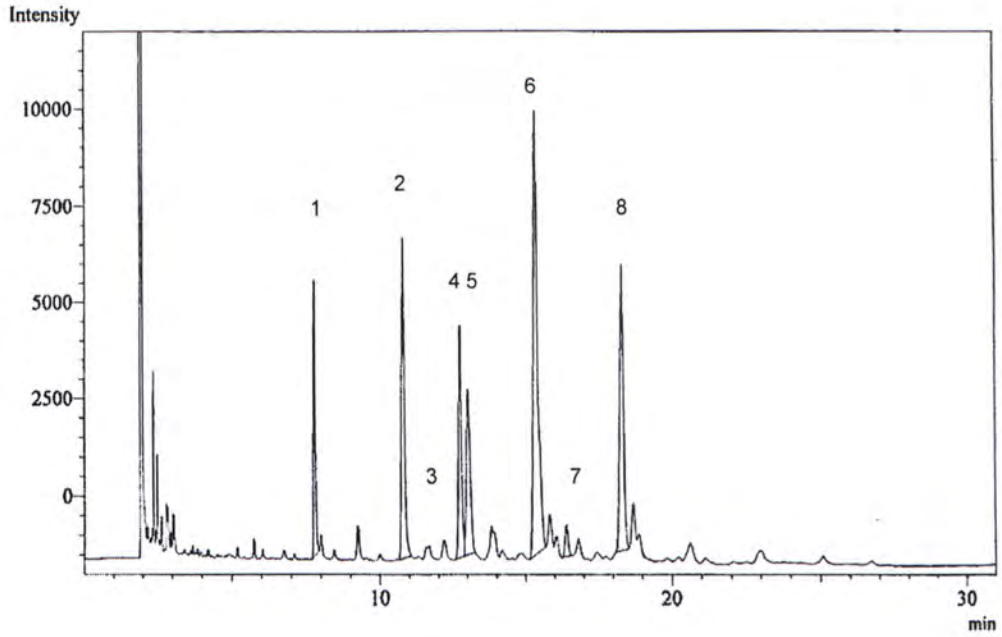


Figure 3.7

Gas liquid chromatogram of fecal neutral sterols of hamsters fed corn oil.

Identification of peaks:

1, 5α-cholestane (Internal Standard); **2**, Coprostanol; **3**, Coprostanone; **4**, Cholesterol; **5**, Dihydrocholesterol; **6**, Campesterol; **7**, Stigmasterol; **8**, β-sitosterol;

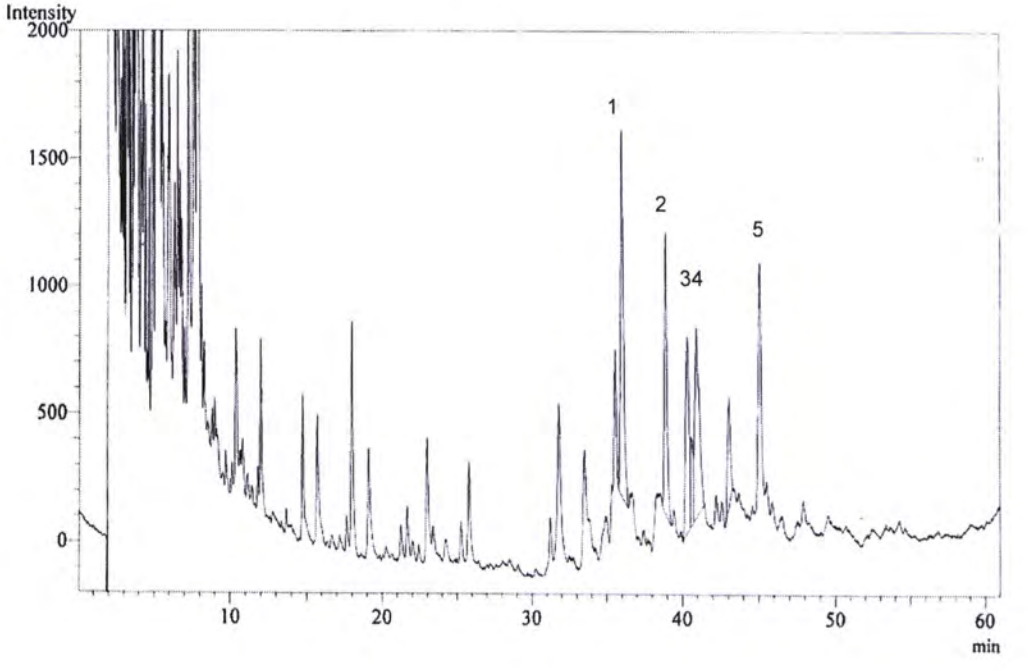


Figure 3.8

Gas liquid chromatogram of acidic sterols in feces. Identification of peaks:

1, Lithocholic acid; **2**, Deoxycholic acid; **3**, Cholic acid; **4**, Hyodeoxycholic acid (Internal Standard); **5**, Ursodeoxycholic acid;

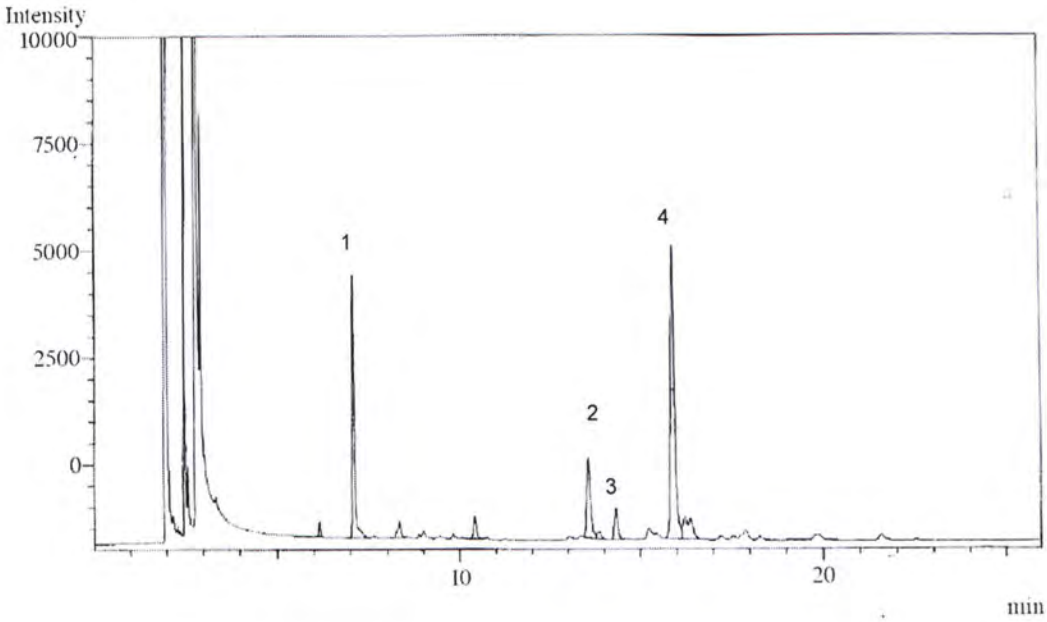


Figure 3.9

Gas liquid chromatogram of phytosterols in corn oil.

Identification of peaks:

1, 5α-cholestane (Internal Standard); **2**. Campesterol; **3**, Stigmasterol; **4**, β-sitosterol;

3.8 Determination of composition and concentration of liver triglycerides, total free fatty acids and phospholipids.

Total lipids of liver were extracted using chloroform-methanol (2:1, vol/vol). 1 mg triheptadecanoate (Sigma Chemical Co., St. Louis, MO, USA) was added as an internal standard to quantify triglycerides (Ringseis et al.) while 1 mg L-phosphatidylcholine diheptadecanoyl (Sigma Chemical Co., St. Louis, MO, USA) was added as an internal standard to quantify total phospholipids (PL) with 0.05 mg triheptadecanoate (Sigma Chemical Co., St. Louis, MO, USA) as internal standard for total free fatty acids (FFA) quantification. Neutral lipid thin-layer chromatography (TLC, 20 x 20 cm plates precoated with 250 μm silica gel 60A, Macherey-Nagel Gmbh & Co. KG, Düren, Germany) was applied to separate total phospholipids (PL), TG, FFA and cholesterol by using a developing solvent system of hexane-diethyl ether-acetic acid (80:20:1, vol/vol/vol). The uppermost band is cholesterol ester, followed by TG, FFA. PL will remain at the starting line. The band of triglyceride, free fatty acids and phospholipids were recovered from the TLC plate, and their fatty acids were converted to the corresponding methyl esters using a mixture of 2 mL 14% boron trifluoride (BF_3) in methanol (Sigma Chemical, St Louis, MO, USA) and 1 mL toluene under nitrogen gas at 100°C for 4 min. The fatty acid methyl esters were extracted with 3 mL hexane and 1 mL H_2O. The upper layer is collected after centrifuge (3000 rpm for 10 min), and was dried under a gentle stream of nitrogen and 0.35 mL hexane was added. The samples were later transfer to GC vials subjected to GC analysis.

The fatty acid methyl esters were analyzed in a flexible silica capillary column (Innowax 19091N-213, 30m x 0.32mm i.d., Alltech, Inc., USA) in a Shimadzu GC 2010 gas chromatograph equipped with a flame-ionization detector (Palo Alto, CA, USA). Column temperature was programmed from 150 to 200°C at a rate of 15°C/min and then gradually increased from 200 to 250°C at the rate of 2°C/min, after which was maintained at 250°C for 5 min. Injector and detector temperature were set at 220°C and 270°C respectively. Helium was used as the carrier gas at a head pressure of 15 psi. Identification of each fatty acid methyl ester was made by comparison of retention time of authentic standards (Sigma Chemical Co., St. Louis, MO, USA). Fatty acids were quantified based on their peak areas. A typical chromatogram is shown in **Figure 3.10**.

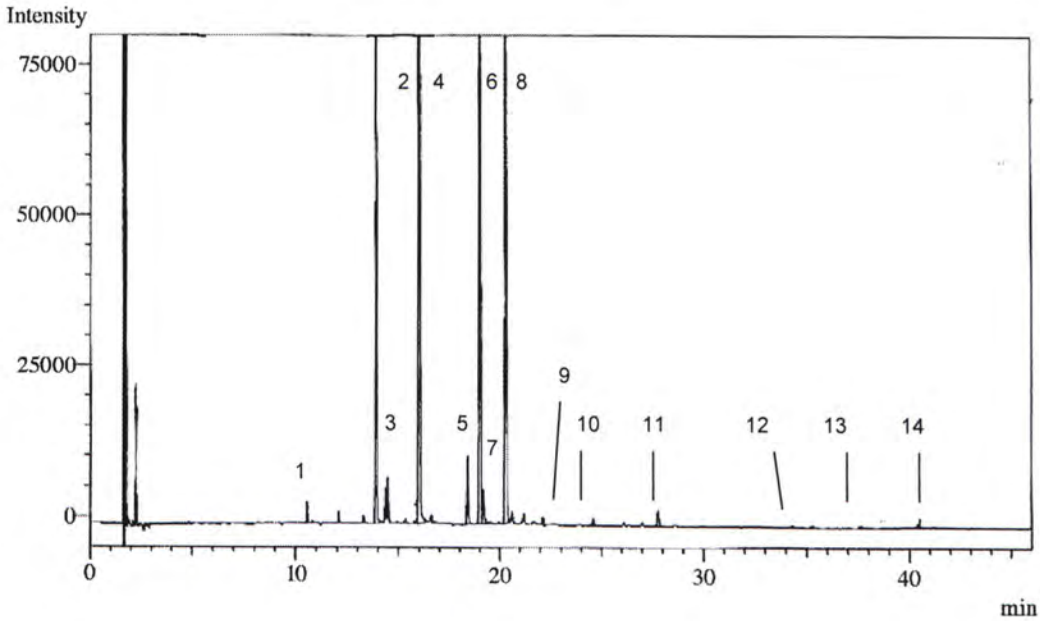


Figure 3.10

Gas liquid chromatogram of hepatic triglycerides in hamster. Identification of peaks:

1, 14:0; **2**, 16:0; **3**, 16:1; **4**, 17:0 (Internal Standard); **5**. 18:0; **6**. 18:1(n-9); **7**. 18:1 (n-7); **8**. 18:2(n-3); **9**. 18:3 (n-3); **10**. 20:1 (n-7); **11**. 20:4 (n-6); **12**. 22:4 (n-6); **13**. 22:5 (n-3); **14**, 22:6 (n-3).

3.9 Statistics

Results were presented as means ± standard deviation (SD). Where applicable, statistical significance of differences between groups was assessed by one-way analysis of variance (ANOVA) followed by Student's *t*-test, or Pearson's correlation coefficient, using Prism® (Graphpad software, Inc., CA, USA). Transformations were used to normalize the data when appropriate and are so indicated. Differences between groups were considered significant when $P<0.05$.

Chapter 4

Results in fried lard experiment

4.1 Fatty acid composition and cholesterol content of experiment lard

The major fatty acid composition of experiment lard and their cholesterol content are shown in **Table 4.1.** With the increasing heat treatment time, polyunsaturated fatty acids (PUFA) significantly and substantially decreased, while saturated fatty acid (SFA) increased significantly, resulting in the significantly decreased PUFA to SFA ratio. The cholesterol concentrations in lard decreased by 18.95%, 22.11%, 30.53% after 2d, 4d, 6d of frying respectively.

4.2 Body weight and food intake

The body weight and food intake of the hamsters are shown in **Table 4.2**. No significant difference in food intake or body weight was observed among the four groups.

4.3 Relative organ weight

Weights of heart, kidney, liver and adipose tissues (epididymal and perirenal fat) of the hamsters are shown in **Table 4.3**.

Table 4.1 Fatty acid composition and cholesterol content of non-fried and fried lard samples.

Fatty acid (%)	NFL	2FL	4FL	6FL
C12:0*	0.32+0.00	0.32+0.00	0.34+0.07	0.34+0.04
C14:0	1.73 ± 0.06^{a}	1.93 ± 0.12^{ab}	1.99 ± 0.07^{b}	2.04 ± 0.03^{b}
C16:0	26.64 ± 0.49^{a}	28.66 ± 0.06^{b}	29.62 ± 0.08^{c}	30.82 ± 0.11^{d}
C16:1	2.03 ± 0.06^{ab}	1.95 ± 0.01^{a}	2.03 ± 0.05^{ab}	2.01 ± 0.01^{b}
C17:1	0.39 ± 0.12^{a}	0.15 ± 0.01^{b}	0.20 ± 0.04^{ab}	0.14 ± 0.03^{b}
C18:0	15.14 ± 0.59^{a}	16.82 ± 0.14^{b}	16.72 ± 0.38^{b}	17.33 ± 0.04^{c}
C18:1(n-9)	37.97 ± 0.62^{a}	36.82 ± 0.04^{b}	37.45 ± 0.06^{a}	37.39 ± 0.14^{a}
C18:1(n-7)	3.01 ± 0.08	2.92 ± 0.06	2.93 ± 0.01	2.90 ± 0.09
C18:2 (n-3)	10.91 ± 0.20^{a}	8.62 ± 0.17^{b}	7.10 ± 0.20^{c}	5.55 ± 0.06^{d}
C18:3 (n-3)	0.48 ± 0.03^{a}	0.29 ± 0.01^{b}	0.18 ± 0.02^{c}	0.11 ± 0.01^{d}
C20:0	0.69 ± 0.02^{a}	0.76 ± 0.02^{b}	0.84 ± 0.01^{c}	0.88 ± 0.01^{d}
C20:1 (n-11)	0.35 ± 0.01^{a}	0.28 ± 0.00^{b}	0.23 ± 0.02^{c}	0.18 ± 0.01^{d}
Others	0.31 ± 0.01^{a}	0.32 ± 0.00^{a}	0.33 ± 0.02^{ab}	0.33 ± 0.00^{b}
SFA	44.54 ± 1.01^{a}	48.65 ± 0.26^{b}	49.53 ± 0.35^{c}	51.40 ± 0.14^{d}
MUFA	43.75 ± 0.83^{a}	42.12 ± 0.09^{b}	42.84 ± 0.13^{a}	42.61 ± 0.11^{a}
PUFA	11.40 ± 0.18^{a}	8.91 ± 0.16^{b}	7.29 ± 0.20^{c}	5.65 ± 0.06^{d}
PUFA/SFA	0.26 ± 0.01^{a}	0.18 ± 0.00^{b}	0.15 ± 0.01^{c}	0.11 ± 0.00^{d}
Cholesterol (mg/g lard)	0.95±0.01^{a}	0.77±0.01^{b}	0.74±0.01^{c}	0.66±0.03^{d}

NFL, non-fried lard; 2FL, lard fried for 2 days ; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD (n=3)

*Number of carbon atoms: number of double bonds.

Means at the same row with different superscripts (a, b, c, d) differ significantly at $p<0.05$

Table 4.2 *Body weight and food intake of the hamsters fed the non-fried and fried lard diets.*

	NFL	2FL	4FL	6FL
Initial body weight (g)	124.4 ± 7.3	121.7 ± 11.2	119.5 ± 9.6	120.0 ± 6.1
Final body weight (g)	133.3 ± 9.4	130.6 ± 14.9	126.0 ± 10.2	128.3 ± 9.0
Food intake (g/day)	11.7 ± 0.4	11.6 ± 0.7	11.5 ± 0.4	11.7 ± 0.6

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD, (n=9)

Table 4.3 *Relative organ weights of hamsters fed the non-fried and fried lard diets. (g/100g body weight)*

	NFL	2FL	4FL	6FL
Heart	0.39 ± 0.03	0.39 ± 0.05	0.39 ± 0.03	0.41 ± 0.04
Kidney	0.82 ± 0.03^{a}	0.86 ± 0.03^{b}	0.93 ± 0.05^{c}	0.92 ± 0.03^{c}
Liver	4.17 ± 0.25^{a}	4.19 ± 0.21^{a}	4.41 ± 0.21^{b}	4.34 ± 0.21^{ab}
Epididymal fat	1.68 ± 0.19^{a}	1.59 ± 0.16^{ac}	1.41 ± 0.19^{b}	1.43 ± 0.19^{bc}
Perirenal fat	0.88 ± 0.20	0.90 ± 0.10	0.77 ± 0.16	0.84 ± 0.13

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD (n=9).

Means at the same row with different superscripts (a, b, c) differ significantly at $p<0.05$

Relative kidney weights increased significantly in 4FL and 6FL compared to the control group, except for that there was no difference between 4FL and 6FL. Relative liver weight also tended to increase with increased heating treatment but only 4FL showed significantly higher weight when compared to NFL and 2FL. However, the epididymal fat decrease significantly when comparing the 4FL to NFL and 2FL groups, 6FL group has an significantly higher weight compared to NFL while it also intended to be higher than that of 2FL (P=0.07). No significant difference in weights was observed in heart and perirenal fats.

4.4 Plasma total cholesterol, triglycerides, and HDL cholesterol

Plasma total cholesterol, HDL cholesterol, triglycerides, non-HDL cholesterol and non-HDL cholesterol to HDL cholesterol ratio are shown in **Table 4.4**. At week 6, experimental groups that consumed fried lard had significantly lower level in both plasma total cholesterol and HDL-cholesterol, however there is no difference among groups in non-HDL cholesterol level or non-HDL cholesterol to HDL-cholesterol ratio or triglycerides level.

4.5 Organ cholesterol

Cholesterol concentrations in the liver and the kidney were determined; the results are shown in **Table 4.5**. The 4FL and 6FL had significantly lower hepatic cholesterol levels when compared to 2FL and control group, while all experiment groups showed significantly lower kidney concentrations comparing to NFL.

Table 4.4 *Effect of fried lard on plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol (Non HDL-C), the ratio of Non HDL-C to HDL-C and triglycerides (TG) of the hamsters at week 6*

	NFL	**2FL**	**4FL**	**6FL**
TC(mg/dl)	236.98 ± 23.81[a]	202.99 ± 25.33[b]	204.08 ± 15.48[b]	204.19 ± 15.11[b]
TG(mg/dl)	190.1 ± 50.0	191.0 ± 37.8	177.3 ± 56.9	185.3 ± 30.4
HDL-C(mg/dl)	114.90 ± 9.53[a]	97.55 ± 11.55[b]	97.52 ± 9.63[b]	97.48 ± 7.82[b]
Non HDL-C(mg/dl)	119.65 ± 19.07	108.07 ± 17.57	111.74 ± 19.87	106.98 ± 10.97
Non HDL-C/HDL-C	1.06 ± 0.15	1.08 ± 0.14	1.10 ± 0.17	1.10 ± 0.14

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD (n=9).

Non HDL-C were calculated by subtracting HDL-C from TC

Means at the same row with different superscripts (a, b) differ significantly at $p<0.01$

Table 4.5 *Organ cholesterol level (mg/g organ wet weight)*

	NFL	2FL	4FL	6FL
Liver	71.58±10.96[a]	65.31±12.43[a]	55.12±7.37[b]	53.43±5.83[b]
Kidney	4.06 ± 0.33[a]	3.64 ± 0.27[b]	3.71 ± 0.31[b]	3.75 ± 0.26[b]

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD (n=9).

Means at the same row with different superscripts (a, b) differ significantly at $p<0.05$

4.6 Fecal neutral sterol output

The daily fecal neutral sterol output of hamster at week 6 is shown in **Table 4.6**. Daily cholesterol and total neutral sterol output were significantly higher in 4Fl and 6FLgroups, while no differences occurred among groups on coprosterol or dihydrocholesterol output.

4.7 Fecal acidic sterol output

The daily fecal acidic sterol output of hamsters at week 6 is shown in **Table 4.7**. 6FL group showed higher excretion of acidic sterol in all types of acidic sterol except ursodeoxycholic acid when compared to the control group (NFL). Substantially increased excretion of deoxycholic acid was found between experimental groups and control group (NFL). And only 4FL and 6FL showed significantly higher total acidic sterol output when compared to the control group.

4.8 Effect of fried lard on cholesterol balance in hamsters

Total intake of cholesterol by the hamsters was compared with its excretion in neutral and acidic sterols and shown in **Table 4.8**. Though 6FL consumed higher amount of cholesterol comparing to NFL, by increased excretion of neutral sterol and acidic sterol, it managed to get a significantly lower cholesterol retention rate when compared with the control group.

Table 4.6 Daily fecal neutral sterol output (mg) in hamsters fed the non-fried and fried lard diets in week 6.

	NFL	2FL	4FL	6FL
Coprosterol	0.830 ± 0.210	0.874 ± 0.290	1.061 ± 0.331	1.230 ± 0.455
Cholesterol	0.474 ± 0.270[a]	0.419 ± 0.160[a]	1.039 ± 0.379[b]	0.858 ± 0.158[b]
Dihydrocholesterol	0.330 ± 0.064	0.260 ± 0.035	0.321 ± 0.040	0.289 ± 0.062
Total Neutral Sterol	1.635 ± 0.354[a]	1.554 ± 0.351[a]	2.421 ± 0.572[b]	2.377 ± 0.573[b]

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD (n=5)

Means at the same row with different superscripts (a, b) differ significantly at $p<0.05$

Table 4.7 Daily fecal acidic sterol output (mg) in hamsters fed the non-fried and fried lard diets in week 6.

	NFL	2FL	4FL	6FL
Lithoxycholic Acid	0.522 ± 0.205^{a}	0.864 ± 0.537^{a}	0.831 ± 0.305^{a}	2.093 ± 0.627^{b}
Deoxycholic Acid	0.240 ± 0.136^{a}	0.582 ± 0.273^{b}	0.540 ± 0.204^{b}	1.366 ± 0.396^{c}
Cholic Acid	0.373 ± 0.260^{a}	0.904 ± 0.856^{ab}	0.628 ± 0.163^{a}	1.563 ± 0.478^{b}
Ursodeoxycholic Acid	0.082 ± 0.025	0.073 ± 0.061	0.117 ± 0.049	0.116 ± 0.114
Total Acidic Sterol	1.217 ± 0.609^{a}	2.422 ± 1.522^{ab}	2.115 ± 0.566^{b}	5.138 ± 1.344^{c}

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD (n=5)

Means at the same row with different superscripts (a, b, c) differ significantly at $p<0.05$.

Table 4.8 Dietary and fecal cholesterol balance in hamsters fed the non-fried and fried lard diets per day per hamster in week 6.

	NFL	2FL	4FL	6FL
Dietary cholesterol intake (mg)	8.16±0.70^{a}	8.81±0.62^{ab}	8.56±0.57^{ab}	9.46±0.53^{b}
Fecal neutral sterol output (mg)	1.64 ± 0.35^{a}	1.55 ± 0.35^{a}	2.42 ± 0.57^{b}	2.38 ± 0.57^{b}
Fecal acidic sterol output (mg)	1.28 ± 0.61^{a}	2.42 ± 1.52^{ab}	2.16 ± 0.57^{b}	5.14 ± 1.34^{c}
Cholesterol retained (mg)	5.31±0.85^{a}	4.84±1.92^{ab}	4.03±0.66^{b}	2.14±1.67^{b}
Cholesterol retained/Cholesterol intake (%)	65.00±8.52^{a}	54.47±20.40^{ab}	47.02±6.94^{b}	22.42±16.83^{c}

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as means ± SD (n=5~6).

Means at the same row with different superscripts (a, b, c) differ significantly at $p<0.05$

4.9 Effect of fried lard on hepatic triglycerides, free fatty acids and phospholipids concentration in hamsters.

As can be seen from **Figure 4.1**, ingestion of fried lard caused decreased hepatic triglycerides when compared with the NCO, and increased hepatic free fatty acid in experimental groups even though there was no difference between 4FCO and 6FCO. The phospholipids concentration of 6FCO was significantly lower than the other three groups. The fatty acid composition of liver triglycerides, free fatty acids and phospholipids are shown in **Table 4.9**, **4.10**, **4.11**.

Regarding the fatty acid composition of hepatic triglycerides, palmitic acid and total SFA was significantly elevated in 4FL and 6FL when compared to NFL and 2FL. Oleic acid (C18:1(n-9)) increased while linoleic acid increased steadily in experiment groups (2FL, 4FL and 6FL). With the reduction in PUFA level in experiment groups, the PUFA/SFA ratio decreased markedly in 4FL and 6FL.

The major fatty acid palmitic acid in hepatic FFA increased in 6FL when compared to other groups. Somehow, there was a variation of C18:1(n-9) in experiment groups. Linoleic acid decreased significantly in 2FL, 4FL and 6FL when compared to NFL. But when all added up together, the total SFA was significantly higher in 6FL and the PUFA together with the PUFA/SFA ratio was significantly lower in 6FL when compared to the other three groups.

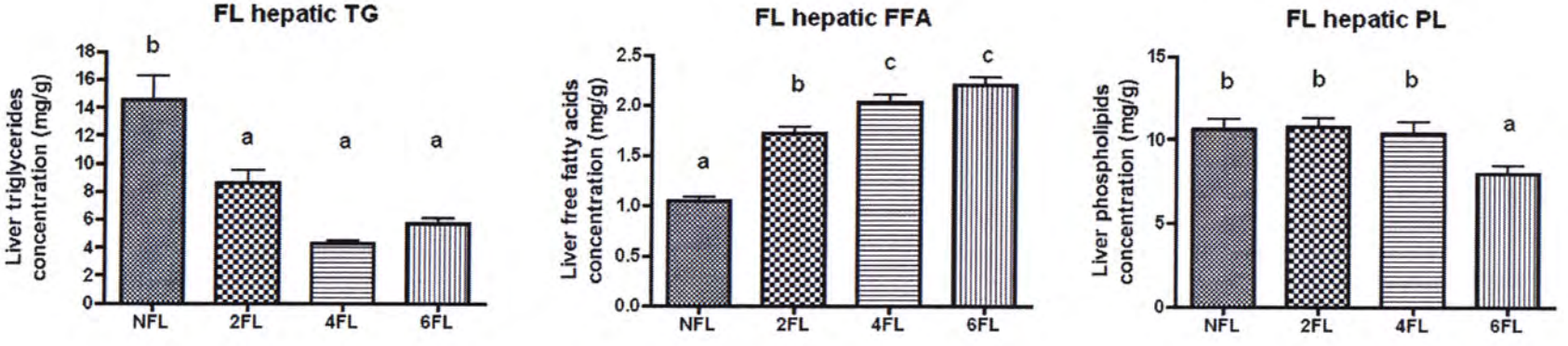


Figure 4.1 Effect of fried lard on liver triglycerides, free fatty acids and phospholipids concentrations.

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Data are expressed as means ± SD (n=9). Means with different letters (a, b, c) differ significantly at $P<0.05$

Table 4.9 Fatty acid composition of hepatic triglycerides of hamsters fed the non-fried and fried lard diets (%).

	NFL	2FL	4FL	6FL
C14:0	0.940 ± 0.176[a]	0.759 ± 0.110[b]	0.700 ± 0.131[b]	0.686 ± 0.202[b]
C16:0	22.956 ± 1.591[a]	23.022 ± 1.865[a]	24.563 ± 1.109[b]	25.248 ± 1.722[b]
C16:1 (n-9)	1.210 ± 0.249	1.152 ± 0.141	1.062 ± 0.115	1.114 ± 0.107
C16:1 (n-7)	2.846 ± 0.393[a]	2.380 ± 0.306[b]	2.051 ± 0.205[c]	1.998 ± 0.158[c]
C18:0	3.548 ± 1.208	3.626 ± 0.596	4.002 ± 0.448	4.369 ± 0.963
C18:1 (n-9)	51.123 ± 1.713[a]	53.362 ± 1.892[b]	53.495 ± 1.902[b]	54.342 ± 2.048[b]
C18:1 (n-7)	2.460 ± 0.247[a]	2.360 ± 0.109[a]	2.206 ± 0.131[b]	2.210 ± 0.126[b]
C18:2 (n-3)	12.919 ± 1.270[a]	11.202 ± 1.001[b]	9.729 ± 1.279[c]	8.145 ± 0.897[d]
C18:3 (n-3)	0.330 ± 0.090[a]	0.237 ± 0.038[b]	0.172 ± 0.054[c]	0.160 ± 0.048[c]
C20:1 (n-9)	0.584 ± 0.085	0.602 ± 0.083	0.561 ± 0.084	0.561 ± 0.071
C20:4 (n-6)	0.748 ± 0.227[a]	0.946 ± 0.249[ab]	1.195 ± 0.251[c]	0.955 ± 0.231[b]
C22:6 (n-3)	0.337 ± 0.100[a]	0.350 ± 0.060[a]	0.264 ± 0.079[b]	0.212 ± 0.060[b]
SFA	27.444 ± 2.397[a]	27.407 ± 2.252[a]	29.265 ± 1.363[b]	30.303 ± 2.232[b]
MUFA	58.222 ± 1.924[a]	59.858 ± 2.100[ab]	59.375 ± 2.104[ab]	60.225 ± 2.193[b]
PUFA	14.334 ± 1.357[a]	12.735 ± 1.125[b]	11.360 ± 1.578[c]	9.472 ± 1.080[d]
PUFA/SFA	0.528 ± 0.080[a]	0.469 ± 0.066[a]	0.389 ± 0.055[b]	0.315 ± 0.048[c]

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as mean ± SD, n=11~12;

*Number of carbon atoms: number of double bonds;

Means at the same row with different superscripts (a, b, c, d) differ significantly;

Table 4.10 Fatty acid composition of hepatic free fatty acids of hamsters fed the non-fried and fried lard diets (%).

	NFL	2FL	4FL	6FL
C14:0	0.570 ± 0.131[a]	0.487 ± 0.060[ab]	0.464 ± 0.052[b]	0.426 ± 0.151[b]
C16:0	26.026 ± 1.585[a]	24.555 ± 1.204[b]	25.177 ± 1.169[ab]	27.861 ± 1.713[c]
C16:1 (n-9)	1.168 ± 0.143[a]	1.080 ± 0.086[ab]	1.072 ± 0.066[b]	1.065 ± 0.086[b]
C16:1 (n-7)	2.506 ± 0.179[a]	2.182 ± 0.138[b]	2.049 ± 0.180[b]	1.682 ± 0.199[c]
C18:0	8.845 ± 1.543[a]	8.850 ± 0.693[a]	9.275 ± 0.977[a]	12.531 ± 2.970[b]
C18:1 (n-9)	39.217 ± 2.639[a]	41.198 ± 1.695[b]	41.309 ± 1.630[b]	38.637 ± 2.403[ac]
C18:1 (n-7)	2.487 ± 0.154[a]	2.350 ± 0.133[b]	2.142 ± 0.175[c]	2.112 ± 0.112[c]
C18:2 (n-3)	12.019 ± 0.777[a]	10.723 ± 0.706[b]	10.037 ± 0.848[c]	7.860 ± 0.954[d]
C18:3 (n-3)	0.353 ± 0.079[a]	0.342 ± 0.564[ab]	0.177 ± 0.083[b]	0.246 ± 0.186[ab]
C20:1 (n-9)	0.462 ± 0.090[a]	0.470 ± 0.063[a]	0.444 ± 0.083[a]	0.328 ± 0.045[b]
C20:4 (n-6)	3.753 ± 0.680[a]	5.063 ± 0.480[b]	5.468 ± 0.355[c]	4.851 ± 0.615[b]
C22:4 (n-6)	0.214 ± 0.048[ab]	0.243 ± 0.044[a]	0.212 ± 0.035[b]	0.198 ± 0.031[ab]
C22:5 (n-6)	0.441 ± 0.097[a]	0.525 ± 0.068[b]	0.472 ± 0.065[ab]	0.518 ± 0.079[b]
C22:5 (n-3)	0.273 ± 0.017[b]	0.298 ± 0.050[b]	0.279 ± 0.046[ab]	0.251 ± 0.025[a]
C22:6 (n-3)	1.666 ± 0.345	1.634 ± 0.318	1.422 ± 0.193	1.479 ± 0.260
SFA	35.441 ± 2.700[a]	33.891 ± 1.727[a]	34.917 ± 1.748[a]	40.818 ± 3.699[b]
MUFA	45.840 ± 2.836[ab]	47.280 ± 1.839[b]	47.016 ± 1.933[b]	43.823 ± 2.718[a]
PUFA	18.719 ± 1.275[a]	18.828 ± 1.405[a]	18.067 ± 1.232[a]	15.359 ± 1.607[b]
PUFA/SFA	0.531 ± 0.057[a]	0.558 ± 0.058[a]	0.519 ± 0.047[a]	0.381 ± 0.061[b]

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL,

fried lard for 6 days.

Values are expressed as mean ± SD, n=11~12;

*Number of carbon atoms: number of double bonds;

Means at the same row with different superscripts (a, b, c, d) differ significantly;

NFL, non-fried lard; 2FL,fried lard for 2 days; 4FL, fried lard for 4 days; 6FL, fried lard for 6 days.

Values are expressed as mean ± SD, n=11~12;

*Number of carbon atoms: number of double bonds;

Means at the same row with different superscripts (a, b, c, d) differ significantly;

Table 4.11 Fatty acid composition of hepatic phospholipids of hamsters fed the non-fried and fried lard diets (%).

	NFL	2FL	4FL	6FL
C14:0	0.123 ± 0.021[a]	0.104 ± 0.015[b]	0.084 ± 0.016[c]	0.076 ± 0.017[c]
C16:0	23.139 ± 1.607[a]	24.494 ± 1.169[b]	22.674 ± 1.630[a]	24.946 ± 1.829[b]
C16:1 (n-9)	0.216 ± 0.025	0.216 ± 0.025	0.224 ± 0.022	0.236 ± 0.037
C16:1 (n-7)	1.101 ± 0.065[c]	1.034 ± 0.113[bc]	0.930 ± 0.097[a]	0.974 ± 0.137[ab]
C18:0	24.596 ± 2.538[a]	25.402 ± 2.078[a]	24.716 ± 2.432[a]	27.891 ± 1.219[b]
C18:1 (n-9)	19.314 ± 1.586[b]	20.513 ± 1.325[bc]	21.078 ± 1.269[ac]	22.634 ± 2.237[a]
C18:1 (n-7)	2.296 ± 0.083[a]	2.000 ± 0.153[b]	1.816 ± 0.169[c]	1.763 ± 0.127[c]
C18:2 (n-3)	12.787 ± 0.913[a]	11.516 ± 0.919[b]	11.427 ± 1.024[b]	9.468 ± 1.013[c]
C18:3 (n-3)	0.165 ± 0.047	0.178 ± 0.089	0.143 ± 0.077	0.147 ± 0.055
C20:1 (n-9)	0.508 ± 0.061[a]	0.393 ± 0.056[b]	0.343 ± 0.042[c]	0.401 ± 0.070[b]
C20:3 (n-9)	1.614 ± 0.334[b]	1.673 ± 0.263[b]	2.042 ± 0.314[a]	1.606 ± 0.342[b]
C20:4 (n-6)	8.899 ± 1.696[c]	8.419 ± 1.192[bd]	10.060 ± 1.939[ac]	7.174 ± 2.295[b]
C22:4 (n-6)	0.267 ± 0.049[a]	0.253 ± 0.041[a]	0.274 ± 0.050[a]	0.182 ± 0.053[b]
C22:5 (n-6)	1.296 ± 0.355[a]	1.103 ± 0.285[a]	1.162 ± 0.322[a]	0.724 ± 0.262[b]
C22:5 (n-3)	0.175 ± 0.034[a]	0.136 ± 0.020[a]	0.135 ± 0.031[a]	0.082 ± 0.021[b]
C22:6 (n-3)	3.504 ± 1.024[b]	2.565 ± 0.560[c]	2.893 ± 0.855[bc]	1.695 ± 0.834[b]
SFA	47.857 ± 3.722[a]	50.000 ± 2.956[a]	47.474 ± 3.811[a]	52.914 ± 2.527[b]
MUFA	23.435 ± 1.649[a]	24.156 ± 1.539[ab]	24.391 ± 1.443[ab]	26.008 ± 2.550[b]
PUFA	28.708 ± 3.881[a]	25.844 ± 2.933[a]	28.135 ± 4.181[a]	21.078 ± 4.675[b]
PUFA/SFA	0.608 ± 0.121[a]	0.521 ± 0.089[a]	0.602 ± 0.128[a]	0.403 ± 0.110[b]

Regarding hepatic phospholipids fatty acid composition, stearic acid (C18:0) was higher in 6FL when compared to the other three groups. Oleic acid (C18:1(n-9)) increased in experimental groups. The 6FL had the higher SFA level and lower PUFA level among all groups, resulting in the 6FL being significantly lower in PUFA/SFA ratio.

4.10 Correlation between serum HDL cholesterol and liver cholesterol

As shown in **Figure 4.2**, there is significant and positive correlation between serum HDL cholesterol and liver cholesterol level in hamsters of all groups ($r=0.5123$, $P=0.001$).

4.11 Correlation between serum HDL cholesterol and kidney cholesterol

As shown in **Figure 4.3**, there is significant and positive correlation between serum HDL cholesterol and kidney cholesterol level in hamsters of all groups ($r=0.3799$, $P<0.05$).

4.12 Correlation between serum TG and liver TG

As shown in **Figure 4.4**, serum TG had no significantly correlation with liver TG level in all hamsters ($r= -0.1174$, $P=0.47$).

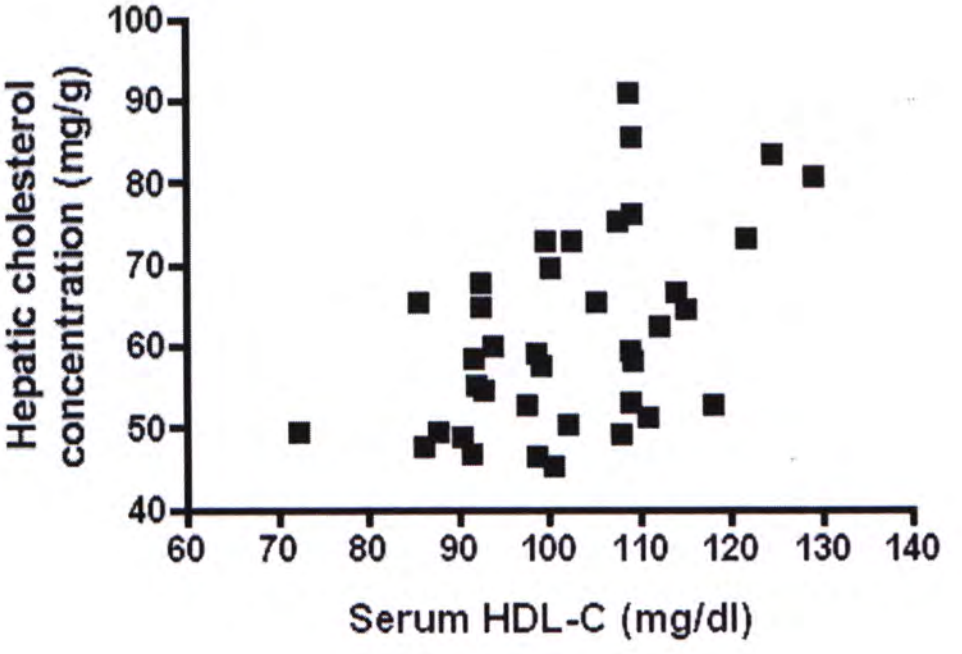


Figure 4.2

Correlation between serum HDL cholesterol level and hepatic cholesterol concentration, n=38, Pearson r=0.5123, P=0.001.

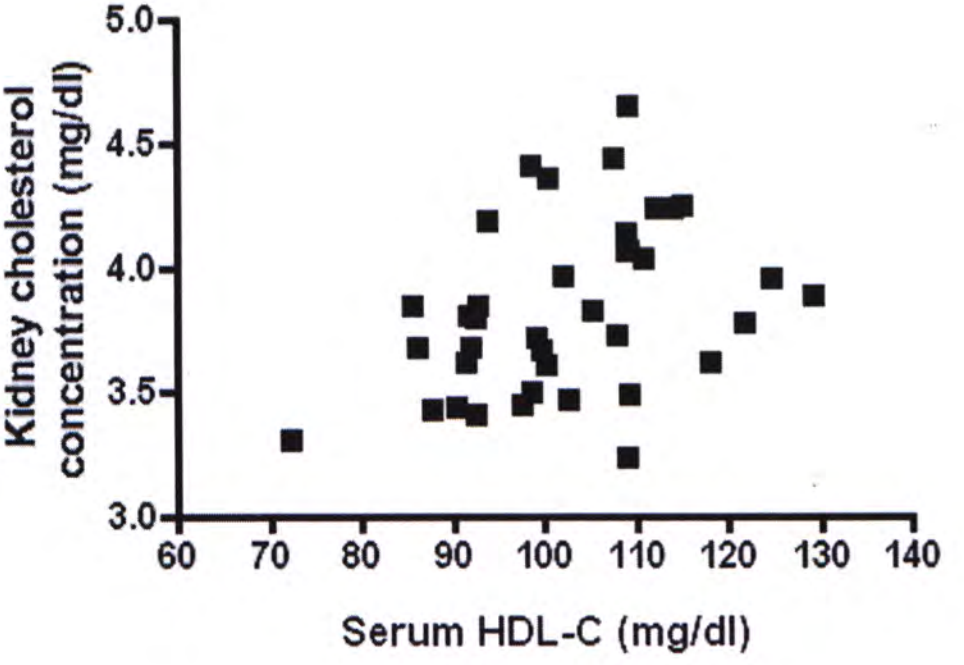


Figure 4.3

Correlation between serum HDL cholesterol level and kidney cholesterol concentration, n=38, Pearson r=0.3799, P=0.019.

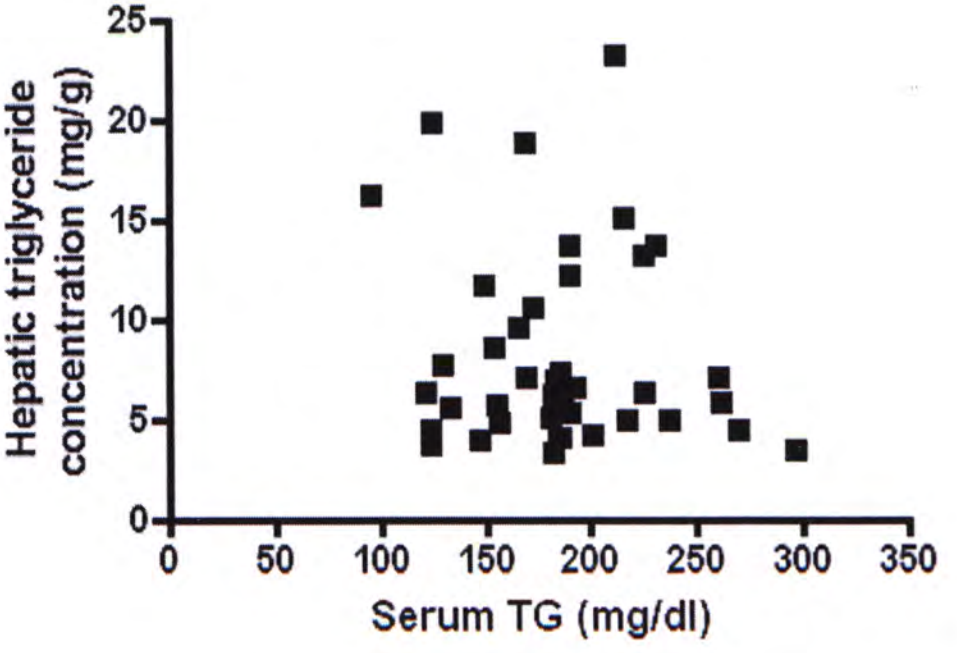


Figure 4.4

Correlation between serum triglyceride level and hepatic triglyceiride concentration, n=38, Pearson r=-0.1174, P=0.47.

Chapter 5

Results of fried corn oil experiment

5.1 Fatty acid composition and phytosterol content of experiment corn oil

The major fatty acid composition of experimental corn oil is shown in **Table 5.1.** The major fatty acids of corn oil are constituted by saturated palmitic acid (C16:0), monosaturated oleic acid (C18:1) and polyunsaturated linoleic acid (C18:2). Except for some insignificant change in 11- octadecenoic acid (C18:1 (n-7)), with increased heat treatment time, all unsaturated fatty acids significantly and substantially decreased, while saturated fatty acids (SFA) increased significantly, resulting in the significantly decreased PUFA to SFA ratio by 42.6% after 6d frying compared to fresh corn oil.

The phytosterol content of experimental corn oils are shown in **Table 5.2**. Although it appeared to be higher in the content of campesterol and beta-sitosterol in 6D corn oil compared to 4D, it is not significant. The 4D and 6D oil samples have markedly lower content of every single phytosterol when compared to 0D and 2D oil samples, as well as in the total amount of phytosterols.

5.2 Body Weight and food intake

The body weight and food intake of the hamsters are shown in **Table 5.3**. No

Table 5.1 Fatty acid composition of non-fried and fried corn oil samples

Fatty acid (%)	NCO	2FCO	4FCO	6FCO
C16:0*	12.624 ± 0.049 [a]	13.251 ± 0.022 [b]	14.525 ± 0.123 [c]	16.702 ± 0.322 [d]
C16:1	0.107 ± 0.002 [a]	0.115 ± 0.001 [b]	0.125 ± 0.002 [c]	0.148 ± 0.005 [d]
C18:0	2.199 ± 0.009 [a]	2.378 ± 0.007 [b]	2.614 ± 0.025 [c]	3.105 ± 0.106 [d]
C18:1 (n-9)	31.518 ± 0.117 [a]	32.759 ± 0.077 [b]	34.427 ± 0.039 [c]	37.481 ± 0.075 [d]
C18:1 (n-7)	1.311 ± 0.138	1.285 ± 0.099	1.373 ± 0.038	1.425 ± 0.029
C18:2 (n-3)	49.385 ± 0.014 [a]	47.356 ± 0.042 [b]	44.074 ± 0.165 [c]	38.349 ± 0.383 [d]
C18:3 (n-3)	1.686 ± 0.070 [a]	1.522 ± 0.026 [b]	1.248 ± 0.006 [c]	0.893 ± 0.013 [d]
C20:0	0.467 ± 0.001 [a]	0.502 ± 0.005 [b]	0.595 ± 0.016 [c]	0.667 ± 0.004 [d]
C20:1 (n-9)	0.109 ± 0.002 [a]	0.189 ± 0.006 [b]	0.314 ± 0.003 [c]	0.452 ± 0.010 [d]
C20:1 (n-7)	0.271 ± 0.001 [a]	0.296 ± 0.009 [b]	0.324 ± 0.008 [c]	0.339 ± 0.019 [c]
C22:0	0.157 ± 0.002 [a]	0.170 ± 0.004 [b]	0.181 ± 0.002 [c]	0.225 ± 0.023 [d]
C24:0	0.164 ± 0.007 [a]	0.178 ± 0.010 [a]	0.200 ± 0.004 [b]	0.214 ± 0.020 [b]
SFA	15.613 ± 0.051 [a]	16.479 ± 0.038 [b]	18.115 ± 0.153 [c]	20.913 ± 0.433 [d]
MUFA	33.317 ± 0.060 [a]	34.644 ± 0.027 [b]	36.563 ± 0.043 [c]	39.846 ± 0.040 [d]
PUFA	51.071 ± 0.084 [a]	48.878 ± 0.049 [b]	45.322 ± 0.170 [c]	39.242 ± 0.396 [d]
PUFA/SFA	3.271 ± 0.015 [a]	2.966 ± 0.010 [b]	2.502 ± 0.031 [c]	1.877 ± 0.057 [d]

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as mean ± S.D (n=3);

*Number of carbon atoms: number of double bonds;

Means at the same row with different superscripts (a, b, c, d) differ significantly at $p<0.05$;

Table 5.2 Phytosterol concentration in non-fried and fried corn oils (mg/g oil)

	NCO	2FCO	4FCO	6FCO
Campesterol	0.545 ± 0.030[a]	0.542 ± 0.029[a]	0.461 ± 0.030[b]	0.467 ± 0.018[b]
Stigmasterol	0.196 ± 0.011[a]	0.179 ± 0.015[ab]	0.165 ± 0.013[b]	0.164 ± 0.010[b]
β-sitosterol	2.170 ± 0.094[a]	2.152 ± 0.116[a]	1.842 ± 0.106[b]	1.878 ± 0.072[b]
Total Phytosterol	2.911 ± 0.132[a]	2.873 ± 0.153[a]	2.469 ± 0.148[b]	2.510 ± 0.094[b]

NCO, non-fried corn oil; 2FCO, corn oil fried for 2 days; 4FCO, corn oil fried for 4 days; 6FCO, corn oil fried for 6 days.

Values are expressed as means ± SD (n=5)

Means at the same row with different superscripts (a, b) differ significantly at $p<0.005$.

Table 5.3 Body weight and food intake of the hamsters fed the non-fried and fried corn oil diets.

	NCO	2FCO	4FCO	6FCO
Initial body weight (g)	116.5 ± 11.56	119.50 ± 7.25	120.50 ± 6.85	119.00 ± 8.76
Final body weight (g)	121.00 ± 8.10	120.50 ± 6.85	125.00 ± 7.07	126.00 ± 6.58
Food intake (g/day)	11.3 ± 0.3	10.9 ± 0.4	11.0 ± 0.3	11.4 ± 0.4

NCO, non-fried corn oil; 2FCO, corn oil fried for 2 days; 4FCO, corn oil fried for 4 days; 6FCO, corn oil fried for 6 days.

Values are expressed as means ± SD (n=10)

significant difference in amount of food intake or body weight was observed among four groups.

5.3 Relative organ weight

Relative weights of heart, kidney, liver and adipose tissues (epididymal and perirenal fat) of the hamsters (g/100g body weight) are shown in **Table 5.4**. Relative liver weight of hamsters increased significantly with longer frying time, except for that there was no difference between 4FCO and 6FCO. Relative kidney weight also increased in heat treatment groups compared to that of NCO. A significantly higher relative heart weight was observed in 4FCO compared to that of NCO. Inversely, the epididymal fat decreased significantly when comparing the 4FCO and 6FCO to NFCO and 2FCO groups. The relative perirenal weight also decreased significantly in group 4FCO and 6FCO compared to the control group.

5.4 Plasma total cholesterol, triglycerides, and HDL-cholesterol

Plasma total cholesterol, HDL cholesterol, triglycerides, non-HDL cholesterol and non-HDL cholesterol to HDL cholesterol ratio are shown in **Table 5.5**. At week 6, 3 experimental groups fed the fried corn oil diets had significantly ($P<0.0005$) lower levels in plasma HDL-cholesterol. However, there was no difference among 4 groups in non-HDL cholesterol level or total plasma cholesterol. But significantly higher level of non-HDL cholesterol to total cholesterol ratio ($P<0.0005$) and serum triglycerides levels ($P<0.005$) occurred in three experimental

Table 5.4 *Relative organ weights of hamsters fed the non-fried and fried corn oil diets. (g/100g body weight)*

	NCO	2FCO	4FCO	6FCO
Heart	0.347 ± 0.015^{a}	0.350 ± 0.021^{ab}	0.362 ± 0.017^{b}	0.356 ± 0.032^{ab}
Kidney	0.749 ± 0.033^{a}	0.866 ± 0.044^{b}	0.869 ± 0.025^{b}	0.894 ± 0.063^{b}
Liver	3.693 ± 0.272^{a}	4.653 ± 0.222^{b}	4.928 ± 0.320^{c}	4.765 ± 0.286^{bc}
Epididymis	1.658 ± 0.204^{a}	1.578 ± 0.216^{a}	1.463 ± 0.109^{b}	1.375 ± 0.267^{b}
Perirenal fat	0.991 ± 0.211^{a}	0.800 ± 0.229^{ab}	0.775 ± 0.153^{b}	0.679 ± 0.197^{b}

NCO, non-fried corn oil; 2FCO, corn oil fried for 2 days; 4FCO, corn oil fried for 4 days; 6FCO, corn oil fried for 6 days.

Values are expressed as mean ± SD (n=10).

Means at the same row with different superscripts (a, b, c) differ significantly at $p<0.05$

Table 5.5 Effect of fried corn oil on plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol (Non HDL-C), the ratio of Non HDL-C to HDL-C and triglycerides (TG)(Ringseis et al.) of the hamsters at week 6.

	NCO	2FCO	4FCO	6FCO
TC(mg/dl)	187.87 ± 23.00	185.98 ± 20.24	182.38 ± 8.34	183.97 ± 13.52
TG(mg/dl)	160.31 ± 65.84^{a}	256.62 ± 75.46^{b}	260.89 ± 60.72^{b}	241.64 ± 47.89^{b}
HDL-C(mg/dl)	81.45 ± 8.47^{a}	68.63 ± 4.70^{b}	69.58 ± 5.02^{b}	66.54 ± 4.83^{b}
Non HDL-C(mg/dl)	106.42 ± 16.80	117.35 ± 18.19	112.79 ± 7.17	117.43 ± 13.11
Non HDL-C/HDL-C	1.307 ± 0.161^{a}	1.711 ± 0.253^{b}	1.629 ± 0.164^{b}	1.775 ± 0.249^{b}

NCO, non-fried corn oil; 2FCO, corn oil fried for 2 days; 4FCO, corn oil fried for 4 days; 6FCO, corn oil fried for 6 days.

Values are expressed as means ± SD (n=10).

Non HDL-C were calculated by subtracting HDL-C from TC

Means at the same row with different superscripts (a, b) differ significantly at $p<0.005$

groups compared to the control group. And there was no difference among the experimental groups in any other profile.

Although there were no differences among groups in serum total cholesterol levels at week 0 and 6, a significantly higher level in 4FCO and 6FCO compared to that in NCO and 2FCO was observed in week 3. However, due to this alternation in TC, serum obtained at week 7 was also tested, TC of 4FCO and 6FCO groups was significantly lower compared to that of NCO. The data are shown in **Figure 5.1**.

5.5 Organ cholesterol

Cholesterol content in liver and kidney have been determined, the results are shown in **Table 5.6**. The 4FCO and 6FCO groups had significantly lower hepatic cholesterol content when compared to 2FCO and the control groups, while only 4FCO and 6FCO showed significantly lower kidney concentrations of cholesterol comparing to NCO.

5.6 Fecal neutral sterol and phytosterol output

The daily fecal neutral sterol (phytosterols excluded) output of hamster at week 6 is shown in **Table 5.7**. At week 6, daily coprostanol and total neutral sterol output were significantly higher in 4FCO and 6FCO groups compared to NCO, while only 6FCO showed a markedly higher excretion of dihydrocholesterol compared to NCO. Coprostanone was only detectable in control group. And there was no difference among groups on cholesterol excretion.

The daily phytosterol output of each hamster at week 6 is shown in **Table 5.8.**

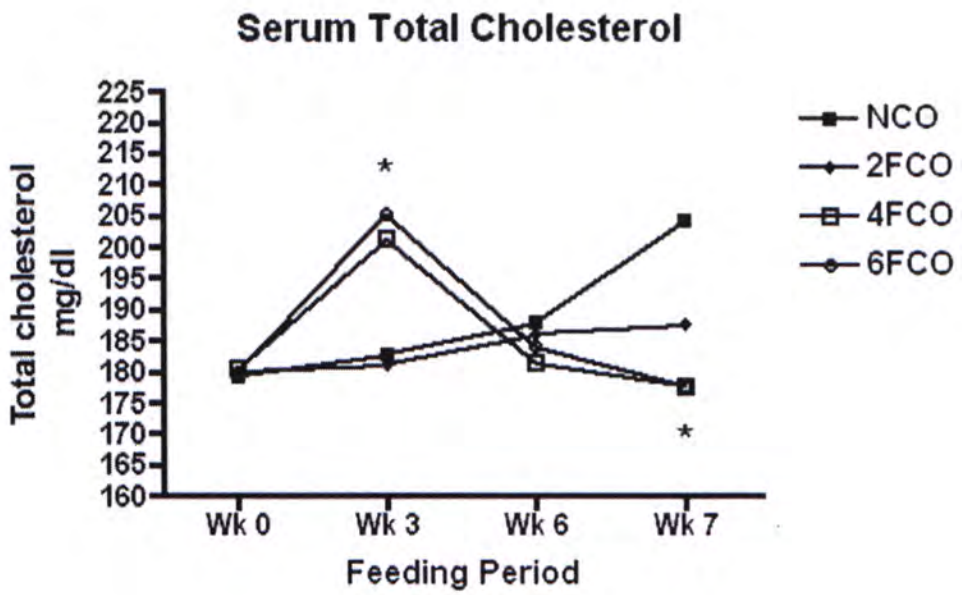


Figure 5.1 Serum total cholesterol levels at week 0, 3, 6 and 7.

Data are expressed as means (n=10).

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

* Difference is considered significant at $P<0.05$ comparing 4FCO and 6FCO with NCO.

Table 5.6 Organ cholesterol level (mg/g organ wet weight)

	NCO	2FCO	4FCO	6FCO
Liver	58.21±10.69[a]	50.32±9.29[a]	34.58±8.45[b]	32.86±6.89[b]
Kidney	3.45 ± 0.14[a]	3.29 ± 0.26[ab]	3.17 ± 0.22[b]	3.31 ± 0.15[b]

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as means ± SD (n=10).

Means at the same row with different superscripts (a, b) differ significantly at $p<0.05$

Table 5.7 Daily fecal neutral sterol output (mg) in hamsters fed the fried non-fried and fried corn oil diets in week 6.

	NCO	2FCO	4FCO	6FCO
Coprosterol	0.288 ± 0.166[a]	0.748 ± 0.299[ab]	1.163 ± 0.471[b]	1.281 ± 0.305[b]
Coprostanon	0.050 ± 0.008	ND	ND	ND
Cholesterol	0.886 ± 0.255	0.922 ± 0.538	1.047 ± 0.243	1.115 ± 0.464
Dihydrocholesterol	0.320 ± 0.052[a]	0.457 ± 0.092[ab]	0.462 ± 0.105[ab]	0.524 ± 0.099[b]
Total Neutral Sterol	1.544 ± 0.467[a]	2.127 ± 0.868[ab]	2.672 ± 0.378[b]	2.921 ± 0.373[b]

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as means ± SD (n=5)

Means at the same row with different superscripts (a, b) differ significantly at $p<0.05$

Table 5.8 Daily phytosterol output (mg) in hamsters fed the non-fried and fried corn oil in week 6.

	NCO	2FCO	4FCO	6FCO
Campesterol	0.990 ± 0.095^{a}	1.360 ± 0.287^{ab}	1.325 ± 0.260^{ab}	1.511 ± 0.287^{b}
Stigmasterol	0.162 ± 0.028^{a}	0.121 ± 0.035^{ab}	0.104 ± 0.029^{b}	0.107 ± 0.027^{b}
β-sitosterol	1.550 ± 0.272	1.163 ± 0.271	1.110 ± 0.281	1.119 ± 0.268
Total Phytosterols	2.702 ± 0.304^{ab}	2.644 ± 0.435^{ab}	2.540 ± 0.084^{a}	2.737 ± 0.049^{b}

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as Means ± SD (n=5)

Means at the same row with different superscripts (a, b) differ significantly at $p<0.05$

There was a decreased output of stigmasterol in 4FCO and 6FCO when compared to the control group. The excretion of campesterol tended to increase from NCO to 6FCO but only excretion in 6FCO was significantly higher than NCO group. The beta-sitosterol output seemed not to be influenced by diet difference. And only significantly higher level of total phytosterol output was shown in 6FCO compared to that in 4FCO.

5.7 Fecal acidic sterol output

The daily fecal acidic sterol output of hamsters at week 6 is shown in **Table 5.9**. The total acidic sterol output varied among groups and no trend was observed at week 6. Only 6FCO was significantly lower in ursodeoxycholic acid excretion when compared to NCO.

5.8 Effect of fried corn oil on cholesterol balance and phytosterol balance in hamsters

Total intake of cholesterol was compared with its excretion in neutral and acidic sterols (**Table 5.10).** Total intake and excretion of phytosterol by the hamsters is shown in **Table 5.11**. It seemed to have decreased in cholesterol retention rate in the three experimental groups but there was no significant difference. But 6FCO had a significantly 49.44% lower phytosterol retaining percentage compared to NCO, while the other 2 groups had the decreasing trend from NCO to 6FCO with no significance compared to any group.

Table 5.9 Daily fecal acidic sterol output (mg) in hamsters fed the non-fried and fried corn oil diets in week 6.

	NCO	2FCO	4FCO	6FCO
Lithoxycholic Acid	0.460 ± 0.286	0.424 ± 0.289	0.698 ± 0.221	0.665 ± 0.352
Deoxycholic Acid	0.201 ± 0.133	0.333 ± 0.117	0.490 ± 0.273	0.554 ± 0.244
Cholic Acid	0.558 ± 0.098	0.626 ± 0.528	0.532 ± 0.199	0.520 ± 0.295
Ursodeoxycholic Acid	0.647 ± 0.364^{a}	0.190 ± 0.163^{ab}	0.185 ± 0.140^{ab}	0.121 ± 0.109^{b}
Total Acidic Sterol	1.866 ± 0.803	1.573 ± 0.830	1.905 ± 0.531	1.860 ± 0.678

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as Mean ± SD (n=5)

Means at the same row with different superscripts (a, b) differ significantly at $p<0.05$.

Table 5.10 Daily dietary and fecal cholesterol balance in hamsters fed the non-fried and fried corn oil diets in week 6

	NCO	2FCO	4FCO	6FCO
Dietary cholesterol intake (mg)	9.00 ± 0.48	8.69 ± 0.70	9.34 ± 0.67	9.57 ± 0.49
Fecal neutral sterol output (mg)	1.54 ± 0.47[a]	2.13 ± 0.87[ab]	2.66 ± 0.40[b]	2.92 ± 0.37[b]
Fecal acidic sterol output (mg)	1.87 ± 0.80	1.57 ± 0.83	2.59 ± 1.61	1.86 ± 0.68
Cholesterol retained (mg)	5.58 ± 1.68	4.99 ± 0.18	4.09 ± 1.63	4.79 ± 1.10
Cholesterol retained / Cholesterol intake (%)	61.55 ± 15.91	57.79 ± 6.94	43.30 ± 15.73	49.87 ± 10.10

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as means ± SD (n=5).

Means at the same row with different superscripts (a, b,) differ significantly at $p<0.05$

Table 5.11 Daily dietary and fecal phytosterols balance in hamsters fed the non-fried and fried corn oil diets in week 6.

	NCO	2FCO	4FCO	6FCO
Dietary Intake (mg)	3.53 ± 0.19^{a}	3.17 ± 0.26^{b}	3.02 ± 0.22^{b}	3.11 ± 0.16^{b}
Fecal output (mg)	2.70 ± 0.30^{ab}	2.64 ± 0.43^{ab}	2.53 ± 0.09^{a}	2.74 ± 0.05^{b}
Phytosterol Retained (mg)	0.83 ± 0.32^{a}	0.52 ± 0.25^{ab}	0.49 ± 0.15^{ab}	0.37 ± 0.15^{b}
Retained/ Intake (%)	23.38 ± 8.51^{a}	16.78 ± 8.72^{ab}	16.11 ± 3.79^{ab}	11.82 ± 4.26^{b}

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as means ± SD (n=5).

Means at the same row with different superscripts (a, b) differ significantly at $p<0.05$

5.9 Effect of fried corn oil on hepatic triglycerides, free fatty acids and phospholipids concentration in hamsters

As can be seen in **Figure 5.2**, all of the three experimental groups which consumed the fried corn oil diets had markedly lower TG level and significantly higher level of FFA when compared to NCO. 4FCO and 6FCO had higher levels of hepatic FFA when compared to 2FCO. There was no difference among the four groups in hepatic PL level.

The fatty acid composition of liver triglyceride, free fatty acids and phospholipids are shown in **Table 5.12, 5.13, 5.14**. The major fatty acids in liver triglycerides were similar to those of the oil sample in the diet. Palmitic acid (C16:0) and oleic acid (C18:1(n-9)) increased significantly in 2FCO, 4FCO and 6FCO when compared to those in NCO, while linoleic acid (C18:2(n-3)) decreased substantially in the experimental groups. The total SFA increased and the PUFA decreased in the experimental groups (2FCO, 4FCO and 6FCO), resulting in the decrease of PUFA/SFA ratio in experiment groups.

The major fatty acids in liver FFA are palmitic acid, oleic acid and linoleic acid. Palmitic acid (C16:0) and linoleic acid (C18:2(n-3)) decreased significantly in experimental groups, but the 6FCO had higher linoleic acid levels when compared to 4FCO. Oleic acid (C18:1(n-9)) increased in the experimental groups. Both of SFA and PUFA decreased significantly in experimental groups. Due to the increase in linoleic acid and other PUFA, the total PUFA of 6FCO was higher than that of 4FCO but still lower than that of NCO.

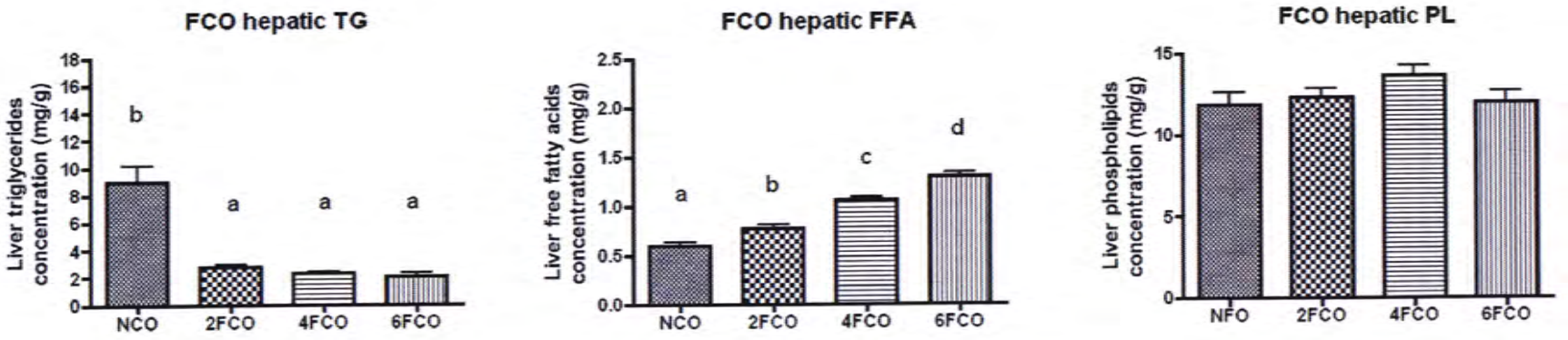


Figure 5.2 Effect of frying corn oil on liver triglycerides, free fatty acid and phospholipids concentrations.

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Data are expressed as means ± SD (n=10). Means with different letters (a, b, c, d) differ significantly at $P<0.05$.

Table 5.12 Fatty acid composition of hepatic triglycerides of hamsters fed the non-fried and fried corn oil diets (%).

	NCO	2FCO	4FCO	6FCO
C14:0*	0.619 ± 0.086^{a}	0.532 ± 0.163^{ab}	0.724 ± 0.211^{ac}	0.482 ± 0.144^{b}
C16:0	20.518 ± 1.607^{a}	24.033 ± 1.335^{b}	25.629 ± 1.175^{b}	24.925 ± 1.676^{b}
C18:0	2.937 ± 0.614^{a}	5.961 ± 2.262^{b}	6.884 ± 1.714^{b}	6.675 ± 1.363^{b}
C18:1 (n-9)	37.658 ± 2.037^{a}	42.204 ± 2.529^{b}	43.605 ± 2.105^{b}	43.012 ± 1.857^{b}
C18:1 (n-7)	1.795 ± 0.130^{a}	1.623 ± 0.567^{ab}	2.051 ± 0.257^{b}	1.927 ± 0.240^{ab}
C18:2 (n-3)	33.641 ± 2.559^{a}	22.735 ± 2.223^{b}	18.048 ± 2.597^{c}	19.343 ± 1.872^{c}
C18:3 (n-3)	0.565 ± 0.074	ND	ND	ND
C20:1 (n-7)	0.430 ± 0.085^{a}	0.501 ± 0.128^{b}	0.380 ± 0.064^{c}	0.518 ± 0.078^{bc}
C20:4 (n-6)	1.133 ± 0.175^{a}	1.653 ± 0.276^{b}	1.832 ± 0.609^{b}	2.207 ± 0.397^{b}
C22:4 (n-6)	0.224 ± 0.081^{a}	0.315 ± 0.062^{b}	0.363 ± 0.071^{b}	0.370 ± 0.095^{b}
C22:5 (n-3)	0.224 ± 0.084^{a}	0.215 ± 0.053^{b}	0.223 ± 0.044^{b}	0.306 ± 0.063^{b}
C22:6 (n-3)	0.256 ± 0.120^{a}	0.205 ± 0.045^{bc}	0.261 ± 0.069^{b}	0.236 ± 0.057^{c}
SFA	24.074 ± 2.020^{a}	30.526 ± 3.408^{b}	33.237 ± 2.637^{b}	32.081 ± 2.667^{b}
MUFA	39.883 ± 2.196^{a}	44.328 ± 2.855^{b}	46.036 ± 2.129^{b}	45.457 ± 1.872^{b}
PUFA	36.043 ± 2.675^{a}	25.145 ± 2.058^{b}	20.727 ± 2.489^{c}	22.462 ± 1.753^{c}
PUFA/SFA	1.512 ± 0.211^{a}	0.836 ± 0.134^{b}	0.631 ± 0.114^{c}	0.707 ± 0.100^{c}

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

*Number of carbon atoms: number of double bonds;

Means at the same row with different superscripts (a, b, c, d) differ significantly.

ND means not detected.

Table 5.13 Fatty acid composition of hepatic free fatty acids of hamsters fed the non-fried and fried corn oil diets (%).

	NCO	2FCO	4FCO	6FCO
C14:0*	0.653 ± 0.306[a]	0.336 ± 0.237[b]	0.466 ± 0.093[ab]	0.058 ± 0.051[c]
C16:0	29.077 ± 1.590[a]	25.255 ± 3.598[b]	25.447 ± 1.325[b]	20.131 ± 1.424[c]
C16:1 (n-9)	1.049 ± 0.124[a]	0.929 ± 0.361[ab]	1.115 ± 0.078[a]	0.731 ± 0.098[b]
C16:1 (n-7)	1.493 ± 0.181[a]	1.077 ± 0.448[b]	1.371 ± 0.102[ac]	0.950 ± 0.137[b]
C18:0	12.119 ± 4.380	11.809 ± 1.804	11.732 ± 1.451	12.539 ± 0.876
C18:1 (n-9)	25.502 ± 1.997[a]	31.651 ± 2.396[b]	33.986 ± 2.211[c]	36.045 ± 1.492[d]
C18:1 (n-7)	1.340 ± 0.158[a]	1.448 ± 0.205[a]	1.516 ± 0.074[b]	1.579 ± 0.066[b]
C18:2 (n-3)	21.593 ± 2.588[a]	18.952 ± 1.791[b]	15.242 ± 1.028[c]	17.576 ± 1.203[d]
C18:3 (n-3)	0.419 ± 0.096[a]	0.259 ± 0.041[b]	0.134 ± 0.058[c]	0.111 ± 0.060[c]
C20:3 (n-9)	ND	ND	ND	1.012 ± 0.113
C20:1 (n-7)	4.555 ± 0.529[a]	6.082 ± 0.720[b]	6.852 ± 0.568[c]	7.195 ± 0.602[c]
C20:4 (n-6)	0.262 ± 0.096[ab]	0.223 ± 0.034[ab]	0.202 ± 0.025[a]	0.229 ± 0.030[b]
C22:4 (n-6)	0.612 ± 0.120[a]	0.412 ± 0.077[b]	0.476 ± 0.070[c]	0.522 ± 0.076[c]
C22:5 (n-3)	0.297 ± 0.065[a]	0.410 ± 0.080[b]	0.380 ± 0.053[b]	0.371 ± 0.069[b]
C22:6 (n-3)	1.029 ± 0.241	1.158 ± 0.255	1.080 ± 0.271	1.044 ± 0.184
SFA	41.849 ± 5.156[a]	37.401 ± 3.474[b]	37.645 ± 2.314[b]	32.727 ± 1.779[c]
MUFA	33.939 ± 2.588[a]	41.186 ± 2.358[b]	44.840 ± 2.298[c]	46.499 ± 1.576[c]
PUFA	24.211 ± 2.818[a]	21.414 ± 1.979[b]	17.515 ± 1.057[c]	20.773 ± 1.341[b]
PUFA/SFA	0.592 ± 0.121[a]	0.581 ± 0.105[ac]	0.467 ± 0.045[b]	0.637 ± 0.065[ac]

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil

for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as mean ± SD, n=11~12;

*Number of carbon atoms: number of double bonds;

Means at the same row with different superscripts (a, b, c, d) differ significantly;

ND means not detected.

Table 5.14 Fatty acid composition of hepatic phospholipids of hamsters fed the non-fried and fried corn oil diets (%).

	NCO	2FCO	4FCO	6FCO
C16:0*	26.345 ± 1.327	26.482 ± 0.784	26.807 ± 1.401	26.702 ± 1.195
C16:1 (n-9)	0.195 ± 0.031	0.211 ± 0.030	0.203 ± 0.030	0.191 ± 0.030
C16:1 (n-7)	0.819 ± 0.078^{a}	0.603 ± 0.068^{b}	0.652 ± 0.057^{b}	0.608 ± 0.058^{b}
C18:0	28.884 ± 3.210^{a}	33.695 ± 1.685^{b}	33.007 ± 1.537^{b}	33.818 ± 1.488^{b}
C18:1 (n-9)	15.433 ± 1.404^{a}	18.215 ± 1.029^{b}	20.711 ± 1.795^{c}	19.484 ± 1.494^{c}
C18:1 (n-7)	1.855 ± 0.172^{a}	1.561 ± 0.089^{b}	1.638 ± 0.189^{b}	1.560 ± 0.105^{b}
C18:2 (n-3)	16.349 ± 1.805^{a}	12.509 ± 0.733^{b}	10.370 ± 0.920^{c}	10.973 ± 0.629^{c}
C20:1 (n-9)	0.470 ± 0.068^{a}	0.400 ± 0.048^{b}	0.408 ± 0.066^{b}	0.382 ± 0.033^{b}
C20:3 (n-9)	0.942 ± 0.202^{a}	1.096 ± 0.123^{b}	1.060 ± 0.183^{ab}	1.131 ± 0.117^{b}
C20:4 (n-6)	5.567 ± 1.874^{a}	3.796 ± 0.520^{b}	3.789 ± 0.518^{b}	3.672 ± 0.573^{b}
C22:4 (n-6)	0.217 ± 0.079^{a}	0.110 ± 0.022^{b}	0.115 ± 0.013^{b}	0.126 ± 0.022^{b}
C22:5 (n-6)	0.935 ± 0.351^{a}	0.265 ± 0.052^{b}	0.282 ± 0.040^{b}	0.295 ± 0.060^{b}
C22:6 (n-3)	1.990 ± 0.960^{a}	1.056 ± 0.124^{b}	0.960 ± 0.149^{b}	1.059 ± 0.133^{b}
SFA	55.229 ± 4.171^{a}	60.177 ± 1.867^{b}	59.814 ± 2.232^{b}	60.520 ± 1.435^{b}
MUFA	18.772 ± 1.649^{a}	20.991 ± 1.056^{b}	23.611 ± 1.951^{c}	22.225 ± 1.584^{c}
PUFA	25.999 ± 4.905^{a}	18.832 ± 1.344^{b}	16.575 ± 1.428^{c}	17.255 ± 1.201^{c}
PUFA/SFA	0.479 ± 0.129^{a}	0.314 ± 0.029^{b}	0.278 ± 0.030^{c}	0.285 ± 0.023^{c}

NCO, non-fried corn oil; 2FCO, fried corn oil for 2 days; 4FCO, fried corn oil for 4 days; 6FCO, fried corn oil for 6 days.

Values are expressed as mean ± SD, n=11~12;

*Number of carbon atoms: number of double bonds;

Means at the same row with different superscripts (a, b, c, d) differ significantly;

In hepatic phospholipids, the major fatty acid C18:0 and C18:1(n-9) increased significantly in experimental groups while C18:2(n-6) decreased in experimental groups. Total SFA increase and PUFA decreased in experimental groups, resulting in decreased PUFA/SFA ratio in experimental groups.

5.10 Correlation between serum HDL cholesterol and liver cholesterol

As shown in **Figure 5.3**, there was significant and positive correlation between serum HDL cholesterol and liver cholesterol level in hamsters of all groups ($r=0.4240$, $P<0.01$).

5.11 Correlation between serum HDL cholesterol and kidney cholesterol

As shown in **Figure 5.4**, serum HDL cholesterol level had no significant correlation with kidney cholesterol level in hamsters of all groups ($r=0.3032$, $P>0.05$).

5.12 Correlation between serum TG and liver TG

As shown in **Figure 5.5**, serum triglyceride level was significantly and negatively correlated with liver TG level in all hamsters ($r=-0.5677$, $P<0.0001$).

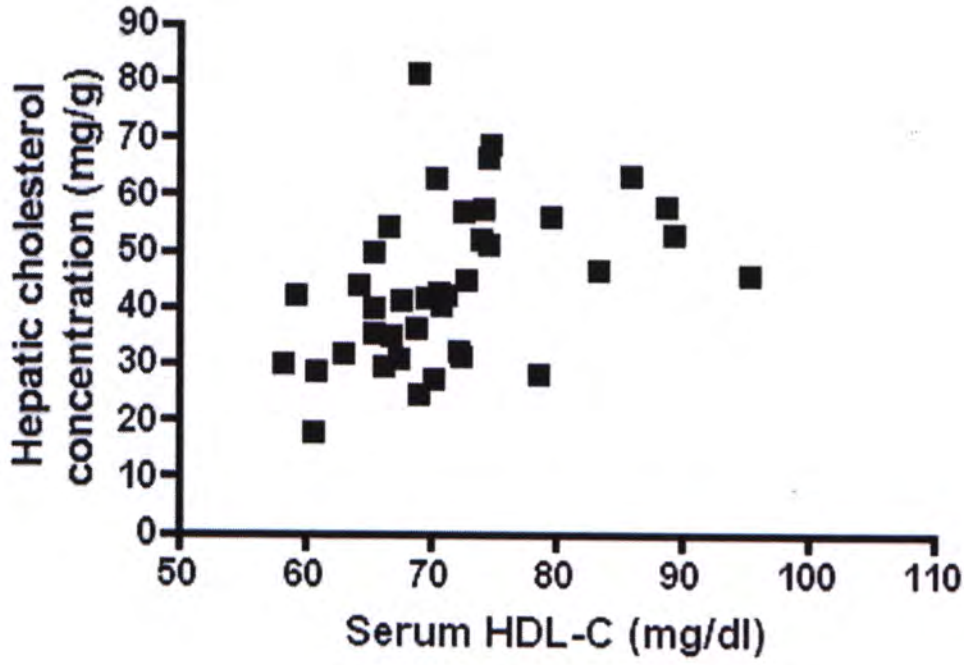


Figure 5.3

Correlation between serum HDL cholesterol level and hepatic cholesterol concentration, n=40, Pearson r=0.4240, P<0.01.

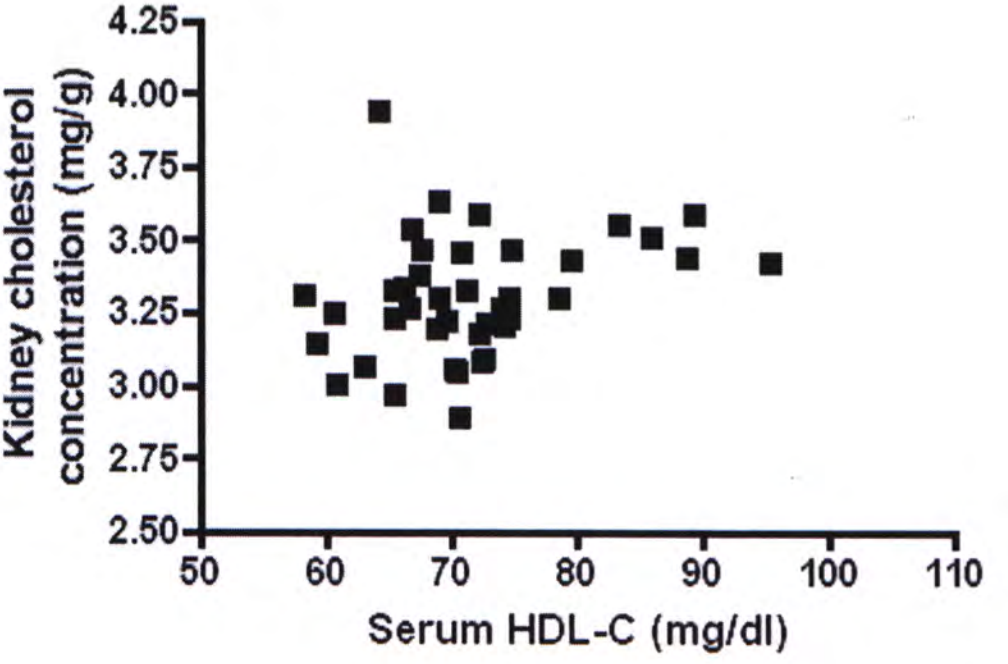


Figure 5.4

Correlation between serum HDL cholesterol level and kidney cholesterol concentration, n=40, Pearson r=0.3032, P=0.06.

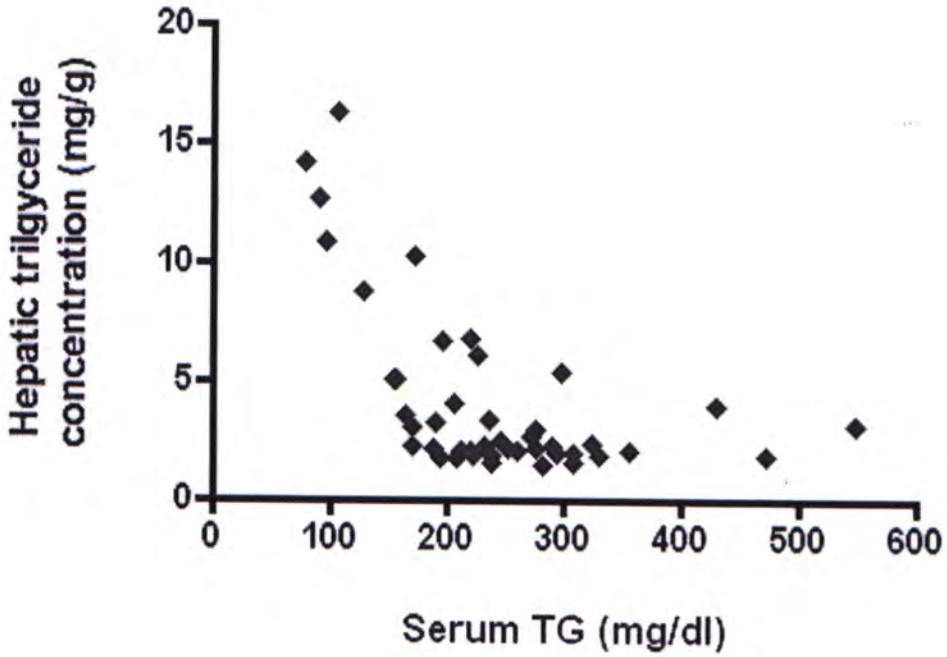


Figure 5.5

Correlation between serum triglyceride level and hepatic triglyceride concentration, n=40, Pearson r=-0.5677, P<0.0001.

Chapter 6

Discussion

The primary aim of this study was to investigate whether and how heat-abused oil influenced the cholesterol metabolism in hamsters. Commercial lard and corn germ oil, as references of both animal fats and vegetable oil, were chosen because of their compatibility to frying due to their different features. We elected the Golden Syrian hamster as a model for human situation since the lipoprotein profile of hamster closely resemble that of humans (Bravo, E., Cantafora *et al.*, 1994). And the hamster has been widely used to study the effect of dietary fatty acids on lipoprotein metabolism and cholesterol profiles (Dietschy *et al.*, 1993; Khosla *et al.*, 1996; Zhang *et al.*, 2002). However, as far as I know of the studies related to my project, hamster has never been used as the animal model to investigate thermoxidized fat on plasma cholesterol level.

The oil undergoes substantial changes in heating treatment. Different temperatures, processing time and some other conditions will result in different amount of primary and secondary peroxidation products. In my study, in order to mimic the heat-abused oil used primarily for frying, heating temperature was set at 180 °C. At high temperature, the primary lipoxy radical is unstable and large quantities of secondary products such as aldehydes or ketones might have been formed (Chang *et al.*, 1978). However, a large portion of relatively volatile peroxidation products are stripped from the oil by air at the high temperature. And a

virtual steady state is reached between their production and removal from the system. Moreover, at high temperature, extensive polymerization and cyclization of fatty acids in the oil will be induced. The formed products in the thermal oxidized fats have been proved to be potent PPARα agonist (Delerive *et al.*, 2000; Konig *et al.*, 2006; Mishra *et al.*, 2004).

It has been reported that feeding of oxidized oils lowers plasma and tissue tocopherol concentrations and causes oxidative stress among rats and pigs (Eder *et al.*, 2003; Liu *et al.*, 1995; Liu *et al.*, 1996; Sulzle *et al.*, 2004; Yoshida *et al.*, 1989). In the two studies, Vitamin E status was not measured. However, the diet used in the studies contain sufficient amount of tocopherols based on the previous studies in our lab and the experimental period was relatively short. I assumed that all the hamsters had an adequate vitamin E intake. Therefore, the results in the two studies are unlikely to be effects of vitamin E deficiency. A cholesterol-related study can last from about 6 weeks to almost 1 year (Guan *et al.*, 2006; McAteer *et al.*, 2003) while the hamsters usually have a lifespan of 2 to 2.5 years (SyrianHamster.com, 2005).

When lard was applied as the experimental fat in the diets, experimental groups which consumed fried lard, regardless of the frying duration, had a significantly lower plasma cholesterol level and HDL-cholesterol level when compared to the control group at both week 3 and 6. However, when corn oil was used, the HDL-cholesterol level was lower in the experimental groups (2FCO, 4FCO and 6FCO), which was consistent with that of the fried lard experiment. But the total cholesterol level in 4FCO and 6FCO first had an increase comparing to NFCO and 2FCO at week 3. The difference disappeared at week 6 then. Due to this

discontinuity, serum was obtained at week 7, when the hamsters were sacrificed. Unexpectedly, a significantly lower serum total cholesterol level occurred in 4FCO and 6FCO compared to the control group, while the HDL-cholesterol level of all groups stayed almost the same as week 6.

Liver and kidney cholesterol levels were measured. In both studies, the groups fed on oil fried for 4D and 6D had a much lower liver cholesterol level when compared to the other 2 groups. While significant difference only occurred between them and the control group in the kidney cholesterol level. Through Pearson's correlation test, only liver cholesterol level was significantly correlated with plasma HDL-cholesterol level. When the data of liver/kidney cholesterol level between the two studies were compared, hamsters that were fed on corn oil had a much lower content of cholesterol in the organs in relative group comparison (i.e. NFL to NFCO, 6FL to 6FCO). Although in these two studies, the hamsters might have come from different strains with similar starting weight to the final point when they were sacrificed, the lard consuming groups had significantly heavier weights compared to the relative group that consumed corn oil. To ensure an indifferent food intake in both groups of hamsters, the food given to each hamster each time was excessive. When the diets were made, food samples were saved for further humidity and other test to calculate a more accurate food intake. By comparison, these two groups had no difference in food intake. Thus the differences that occurred could be more specific from the oil. The relative organ weights were stated previously in detail; however, the two studies only shared some similarity in these profiles. In fried lard experiment, only 4FL and 6FL had significantly higher relative liver weight compared to the control group, but in fried corn oil experiment, all the three

experimental groups had markedly higher relative liver weight when compared to NCO, showing a possible general toxicity of frying oil administration. Similar situation occurred in both experiments in relative kidney weight and the relative heart weight of fried corn oil experiment.

Thinking of the cause of the above organ weight and cholesterol level differences, fatty acid composition of the oil might be the major contributor. In both oils, PUFA decreased and SFA increased with increased frying time. But the lowest content of PUFA in corn oil experiment was as high as about 40% of total fats while the highest PUFA content in the lard experiment was around 10%. Besides, it cannot be neglected that the lard is a cholesterol-containing animal fat, the little difference in the cholesterol that consumed by hamster might have also contributed to the plasma and organ cholesterol level differences. In addition, in both experiments, the fried oil with prolonged frying time had increased viscosity, which implies the accumulation of polymerized product.

In fried lard experiment, by higher excretion of neutral and acidic sterols through feces, the hamsters that consumed prolong-fried lard had a lower cholesterol retained/intake ratio when compared to the control group, partly explaining the decreased total cholesterol level in plasma in experimental groups. At week 6, experimental groups had much higher acidic sterol excretion, which implied possible activation of CYP 7A1.

In fried corn oil experiment, the serum total cholesterol levels were not as consistent as that in fried lard study. Although the PUFA could have been an explanation partly, considering the difference converted into the food intake, the

PUFA intake difference was only about 1% between NCO and 6FCO. Thus assumption was made that the difference in the plasma total cholesterol did not account for the difference in the PUFA content in the oils. And this was further confirmed by lowered hepatic cholesterol levels in experimental groups.

Phytosterol balance was calculated due to abundant content of phytosterols in corn oil and its competitive relationship with cholesterol in cholesterol absorption in the intestine. At week 3, the phytosterols retained/intake ratio in the hamsters did not differ from each group; however, there was an increased neutral sterol output in experimental groups while the acidic sterol concentration in the feces decreased significantly in these groups, suggesting a possible inhibition on CYP 7A1 activity by fried corn oil, which is contradictory to that of fried lard experiment. An insignificant declining trend was obtained from NCO to 6FCO in the cholesterol retained/intake rate at week 3. Such trend was obtained and statistically significant at week 1. Hence, though the cholesterol retained/intake ratio was not significantly different in at week 3, the increased TC in 4FCO and 6FCO at week 3 could be partly explained by the lowered cholesterol retained/intake ratio in the two groups. At week 6, there was a significant decrease in experimental groups in the phytosterol that retained, while indifferent cholesterol retained/intake ratios were obtained.

Interestingly, the plasma TG level in fried corn oil groups were significantly higher when compared to the control group. Thus the liver TG, free fatty acid (FFA) and phospholipids (PL) in both studies were assessed. It is consistent in both studies that the hepatic TG level is significantly lower in the experimental groups when compared to the control group, and a markedly increasing trend of the FFA level was

seen. Although such parameters of experimental oils were not tested, it is true that during frying more FFA will be released from decomposed TG. Hepatic lipid compositions strongly reflected the fatty acid composition of the ingested fat. In corn oil experiment there was no difference among PL levels but in fried lard study a marked lower concentration of PL in 6FL occurred when compared to all other groups. However, it was different in fried lard experiment that there was no difference among groups in plasma TG level.

With the above-mentioned data, it is very much likely that the differences in the cholesterol level or the TG level are a result of activation of PPARα of fried oil. Studies show that by clinical administration of a PPARα agonist, e.g. fibrates, human plasma HDL cholesterol level was elevated by 10% which later translated into a 25% deduction in risk for major coronary diseases (Birjmohun *et al.*, 2005). However, such beneficial effect is not observed in rodents' studies, such as using mice as a model (Peters *et al.*, 1997; Staels *et al.*, 1992). Kersten (Kersten, 2008) suggested that the species-specific regulation of lipoprotein A-I (APOA1) --- the core apolipoprotein of HDL, is likely to possibly explain the differential effects of PPARα on plasma HDL cholesterol between mice and humans. mRNA expression of APOA1 in liver and plasma can be increased by activation of PPARα in humans (Berthou *et al.*, 1996), while opposite effect showed up in rodents (Staels *et al.*, 1992). And this is further proved by findings that 3 nucleotides are different between the rat and the human APOA1 promoter A site, turning it an positive response element to PPARα in humans to a nonfunctioning element in rats (Vu-Dac *et al.*, 1998). Considering genetics building among rats, mice, hamsters and humans, it is

likely that similar situation could be true in hamsters, just like rats, resulting in the decreased HDL-cholesterol level.

Many studies found out with an activation of PPARα, there's always been a lower liver TG level with a fatter liver, which is in agreement with the current study results (Haluzik *et al.*, 2006; Harano *et al.*, 2006; Linden *et al.*, 2001; Linden *et al.*, 2002; Martin, P. G. *et al.*, 2007; Nagasawa *et al.*, 2006; Tordjman *et al.*, 2001). Most of the researches explained the TG-lowering effect in the liver by activation of PPARα is that by promoting fatty acid oxidation.

It is proved that the expression of lipoprotein lipase (LPL) in the liver is up-regulated by PPARα (Schoonjans *et al.*, 1996), but somehow, it appears that PPARα agonist stimulated plasma TG clearance by altering the hepatic expression of inhibitors or activators of LPL activity rather than regulating on LPL itself (Bard *et al.*, 1992; Lemieux *et al.*, 2003; Minnich *et al.*, 2001; Staels *et al.*, 1995). Nevertheless, the TG can also be regulated by SREBP-1 through the post-transcriptional (Hannah *et al.*, 2001) or transcriptional levels (Shimano *et al.*, 1999; Xu *et al.*, 2002), and other possible pathways.

Despite the liver, intestine is another major share of HDL cholesterol synthesis (Brunham *et al.*, 2006; Timmins *et al.*, 2005); neverhterless, mechanism operating in intestine is not clear and thus becomes an emerging research interest. And certain enzymes and protein concentrations can be assessed by empirical methods to elucidate the underlying mechanism (Ayyobi *et al.*, 2000; Graf *et al.*, 2003).

We use the hamsters as a model to study the effects of frying lard and frying corn oil on serum cholesterol level and cholesterol metabolism. Eventually, this model is to obtain information about the human situation. It should be emphasized that when compared with human subjects, there may be differences in response to diet containing frying fats/oil. And the prepared fat/oil used in the two studies does not directly represent the oxidized fat in human nutrition that originates from deep fried foods.

It is mostly discussed in this chapter that the physical alternations in cholesterol levels and TG levels by fried lard and corn oil are the results of changes of fatty acid composition and phytosterol content in the oil due to high temperature process, with suggested relevant pathway by other scientists, results in my experiment could be partly well-explained. But it does not mean that the discussion here can totally account for the results. The cholesterol metabolism in mammalians are complicated, multi-layer regulated by a lot of factors.

In order to make a better comprehension of the full picture of this experiment, I will suggest more experiments be applied to assess the change in transcriptional or post-transcriptional levels as well as experiments to assess more detailed constitution in fried oil samples.

Chapter 7

Conclusion

In conclusion, this study shows that fried lard and corn oil are able to lower plasma HDL-cholesterol and hepatic cholesterol level in hamster, and are able to lower TG production in liver, increase hepatic FFA content while enhance TG clearance through plasma. Explanation of the alternation in different profiles are based on assumption of activation of PPARα by oxidized oil. Hence, related protein concentrations in liver and mRNA level should be assessed in future studies to prove its probability. And in fried corn oil study, the proteins and certain enzymes related to cholesterol absorption in the intestine should be investigated because of the presence of phytosterols.

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